



2014

Microsphere Spray System for Wound Coverage

Nicholas J. Andersen

University of Kentucky, Nicholas.Andersen@uky.edu

Recommended Citation

Andersen, Nicholas J., "Microsphere Spray System for Wound Coverage" (2014). *Theses and Dissertations--Biomedical Engineering*. 23. http://uknowledge.uky.edu/cbme_etds/23

This Master's Thesis is brought to you for free and open access by the Biomedical Engineering at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Biomedical Engineering by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Nicholas J. Andersen, Student

Dr. David A. Puleo, Major Professor

Dr. Abhijit Patwardhan, Director of Graduate Studies

MICROSPHERE SPRAY SYSTEM FOR WOUND COVERAGE

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in Biomedical Engineering
in the College of Engineering
at the University of Kentucky

By

Nicholas John Andersen

Lexington, Kentucky

Director: Dr. David A. Puleo, Professor of Biomedical Engineering

Lexington, Kentucky

2014

Copyright © Nicholas John Andersen 2014

ABSTRACT OF THESIS

MICROSPHERE SPRAY SYSTEM FOR WOUND COVERAGE

Spinal fusion is used to treat diseases or disorders of the spine by fusing together two or more vertebrae. Two associated risks with spinal fusion are infection and blood loss. Administration of tranexamic acid is used to prevent blood loss, and transfusions are given following blood loss. Surgical site infections are prevented with vancomycin powder spread into the surgical wound, while established infections are treated by debridement and delivery of antibiotics for 4 to 6 weeks. The present research explored an alternate method to prevent and treat blood loss or infection in spinal fusion. Poly(lactic-co-glycolic acid) (PLGA) microspheres was used to encapsulate vancomycin for 42 days to treat infection. Vancomycin encapsulated in gelatin microspheres had a controlled release of 7 days to prevent infection. Tranexamic acid was dissolved into phosphate-buffered saline or carboxymethylcellulose to provide a release of 6 hours to prevent blood loss after surgery. The microspheres and tranexamic acid were delivered to a target region using a water based spray system. The spray system demonstrated the delivery and distribution of drugs to a target region. The microsphere spray system is capable of spraying drugs onto a target region to prevent or treat blood loss and infection over time.

KEYWORDS: PLGA, gelatin, microspheres, controlled release, spinal fusion

Nicholas John Andersen

September 23, 2014

MICROSPHERE SPRAY SYSTEM FOR WOUND COVERAGE

By

Nicholas John Andersen

David A. Puleo
Director of Thesis

Abhijit Patwardhan
Director of Graduate Studies

Date

ACKNOWLEDGMENTS

I would like to thank Dr. Puleo for accepting me into his laboratory and giving me this opportunity. Dr. Puleo's wisdom and guidance were invaluable to myself and this research. I would also like to thank my committee members Dr. Milbrandt and Dr. Hilt for their help and expertise in this research.

I would also like to thank my family for all the support and encouragement they have given me during this endeavor. Finally I would like to thank my labmates for all their help and providing a great fun work place in the laboratory.

TABLE OF CONTENTS

MICROSPHERE SPRAY SYSTEM FOR WOUND COVERAGE
ABSTRACT OF THESIS i
ACKNOWLEDGMENTS iii
TABLE OF CONTENTS iv
LIST OF TABLES vi
LIST OF FIGURES vii
1. INTRODUCTION 1
2. BACKGROUND AND SIGNIFICANCE 3
 2.1. Spinal fusion 3
 2.2. Spinal fusion blood loss 3
 2.3. Spinal fusion surgical site infections 3
 2.4. Treatment and prevention of blood loss 4
 2.5. Treatment and prevention of infection 5
 2.6. Vancomycin and tranexamic acid used 6
 2.7. Poly(lactic-co-glycolic acid) 7
 2.8. Gelatin 8
 2.9. Carboxymethylcellulose 9
 2.10. Spray systems 9
 2.11. Significance 10
3. METHODS AND MATERIALS 11
 3.1. Poly(lactic-co-glycolic acid) microspheres 11
 3.2. Gelatin microspheres 12
 3.3. Drug analysis 13
 3.3.1. Vancomycin 13
 3.3.2. Tranexamic Acid 13
 3.4. Tranexamic acid spray solution 13
 3.5. Release studies 14
 3.6. Spray gun 15
 3.7. Spray gun experiments 16
 3.7.1. Fluid medium 16
 3.7.2. Volume per spray 17
 3.7.3. Area coverage 17

3.7.4.	Mass of microspheres per spray	21
3.8.	Drip test	21
3.9.	Statistics	22
4.	RESULTS	23
4.1.	Release studies and loadings	23
4.1.1.	Vancomycin encapsulated PLGA microspheres	23
4.1.2.	Vancomycin loaded in 20 mM crosslinked gelatin microspheres	25
4.1.3.	Tranexamic acid loading in PLGA.....	27
4.1.4.	Tranexamic acid release from gelatin microspheres	27
4.1.5.	Tranexamic release profile from PBS and 1% CMC	29
4.2.	Spray solution.....	30
4.3.	Volume per spray	31
4.4.	Area coverage.....	32
4.5.	Mass of microspheres per spray	34
4.6.	Drip Test.....	35
5.	DISCUSSION.....	37
6.	CONCLUSION	46
7.	REFERENCES	47
8.	VITA.....	53

LIST OF TABLES

Table 4.1: Spray Solution Test.....	31
Table 4.3: Drip rate for different fluid mediums.	36

LIST OF FIGURES

Figure 3.1: Spray gun with 50 ml tube.	16
Figure 3.2: Modified tubing and 50 ml tube connection.	16
Figure 3.3: Area coverage spray testing set up post spray (side view).....	19
Figure 3.4: Area coverage spray testing set up post spray (bird's eye view).....	20
Figure 3.5: Image Analysis Diagram.	20
Figure 3.6: Representation of Drip Test Set Up.	22
Figure 4.1: (A) Instantaneous and (B) cumulative profiles showing release of vancomycin from 75:25 PLGA. Data are mean \pm standard deviation (n = 3). The error bars are shown on Figure 4.1 part B but are hidden by the data points. The standard deviation ranged as high as \pm 89 μ g and low as \pm 7 μ g.	24
Figure 4.2: (A) Instantaneous and (B) cumulative profiles showing release of vancomycin from 20mM crosslinked gelatin. Data are mean \pm standard deviation (n = 3).	26
Figure 4.3: (A) Instantaneous and (B) cumulative profiles showing release of tranexamic acid from 20mM crosslinked gelatin in PBS and 1% CMC. Data are mean \pm standard deviation (n = 3).	29
Figure 4.5: (A) Instantaneous and (B) cumulative profiles showing release of tranexamic acid from 20mM crosslinked gelatin in PBS and 1% CMC. Data are mean \pm standard deviation (n = 3).	30
Figure 4.6: Volume per spray for the spray system. Data are mean \pm standard deviation (n = 3).	31
Figure 4.7: Area coverage for different numbers of sprays. Data are mean \pm standard deviation (n = 3).	32
Figure 4.8: First and fifth sprays for area coverage analysis.	33
Figure 4.9: Cumulative mass of microspheres per spray for gelatin and PLGA microspheres. Data are mean \pm standard deviation (n = 3).	34
Figure 4.10: Mass of microspheres per spray for 1 through 5 sprays for gelatin and PLGA microspheres. Data are mean \pm standard deviation (n = 3).	35

Figure 4.11: Drip test for different fluid mediums. Data are mean \pm standard deviation (n = 3). 36

INTRODUCTION

Spinal fusion is a type of surgery that involves permanently fusing together two or more vertebrae in the spine to stop motion between them. Spinal fusion surgery is often needed to correct conditions from degenerative disc disease, scoliosis, spondylosis, vertebrae fracture and several other degenerative spinal conditions. The surgery frequently requires instrumentation to fix the condition by stabilizing and aligning the spine.

Two major risks of spinal fusion are major blood loss and a postoperative infection. A patient can lose up two liters of blood during and after surgery, which can result in a variety of issues. A postoperative infection in the surgical site results in extended hospital stays, increased patient cost, and other health risks.

Blood loss is treated by having the patient receive blood transfusions. Blood transfusions have associated risks with them such as infection, transmission of blood borne diseases, allergic reaction and delayed hemolytic reaction.

Surgical site infections are treated by administering antibiotics intravenously for an average of 6 weeks, followed by oral antibiotics for several months. To prevent surgical site infections, antibiotics in powder form can be spread into the surgical site. The current method to prevent infections requires intravenous antibiotics daily and requires a large dose of antibiotics spread into the wound. A large amount of antibiotics are lost due to wound drainage. The patient must remain in the hospital for an extended time due to the need for intravenous antibiotics. This increase in hospital care can be a financial burden. There is no current method that has sustained or controlled release of antibiotics which would allow the patient to return home.

This research focused on spinal fusion surgery and the various issues with the current prevention/ treatment options by using biomaterials engineering. Poly(lactic-co-glycolic acid) (PLGA) and gelatin were used as the polymers to deliver antibiotics over extended time to the surgical site. PLGA and gelatin are both biodegradable and nontoxic. PLGA and gelatin were both formed into microspheres to encapsulate vancomycin. Tranexamic acid was suspended in solution to be locally delivered to the surgical site. A water based spray system was utilized to spray either PLGA or gelatin microspheres in a tranexamic acid solution directly onto the surgical site. This would allow for the simultaneous delivery and sustained release of tranexamic acid and/or vancomycin. Therefore, it would treat or prevent blood loss and infection at the same time.

BACKGROUND AND SIGNIFICANCE

2.1. Spinal fusion

Spinal fusion surgeries have been occurring since the early 1900s to treat various diseases or conditions of the spine. Spinal fusion is used to treat deformity, trauma, degenerative disc disease, scoliosis and spondylolisthesis [1]. The surgery is the permanent fixation of two or more vertebrae and often requires the use of instrumentation to stabilize the spine [2]. There are three different sections to the spine that can require fusion; the cervical, thoracic and lumbar curve. From 1998 to 2008 there has been a 114% increase in cervical fusion, an 82% increase in thoracic fusion and 171% increase in lumbar fusion in the United States [1]. It is clear there is an increasing number of spinal fusion required each year, but spinal fusion has associated risks in blood loss and infection.

2.2. Spinal fusion blood loss

A patient can lose up to 2 liters of blood during surgery and up to a liter of blood can be lost postoperatively [3]. To replace the blood lost, a patient will have blood transfusions. The amount of blood lost is directly related to the number of vertebrae fused. For 2-3 vertebrae fused, a patient will have 8.3% of their overall blood transfused. For 4-8 vertebrae fused 18.5% of their overall blood is transfused and ≥ 9 vertebrae fused 37.9% overall of the patient's blood is transfused [4]. In adult spinal fusion, the percentage of transfusions required in patients ranges between 50% and 81% [5]. Blood transfusions have serious risks such as reactions to transfused blood, alloimmunization, transfusion-associated bacterial sepsis and infection risks which include human immunodeficiency virus and hepatitis [3, 5, 6].

2.3. Spinal fusion surgical site infections

Another serious risk of surgery is a surgical site infection (SSI), especially in spinal fusion involving instrumentation. The incidence of infection in spinal fusion surgery involving instrumentation ranges from 2.1% to 8.5% [7]. There are over 500,000 SSIs in America each year which results in an expenditure of billions of dollars [8]. In America there were approximately 415,000 spinal fusion surgeries in 2008. If the incident rate of infection is 8.5% that would result in 35,275 SSIs each year [1]. The mean cost per infection is approximately \$34,000 and the infection related cost per 100 spinal fusions is approximately \$438,000 [8]. Therefore, the total infection related cost of SSIs in spinal fusion is approximately \$154 million dollars per year in the United States. The most predominate organism to cause SSIs is *Staphylococcus aureus*. *S.aureus* was found in 73% of deep SSIs cases and 86% in superficial SSIs [2, 7, 9]. Starting on October 1, 2008 a new Medicare policy was put into place for preventable conditions such as infections. The policy was a reduced payment for these conditions. Therefore, Medicare no longer paid hospitals more for a patient who developed a preventable condition after admission. This meant hospitals and insurance companies would not be covering the cost of a surgical site infections [10, 11]. The patient can no longer expect reimbursement for an infection developed after spinal fusion.

2.4. Treatment and prevention of blood loss

Due to the significant amount of blood loss in spinal fusions there are treatment and preventative methods which are currently being used. To treat blood loss from surgery, a patient will need to have blood transfusions to replace the blood lost [3]. The technique to prevent or reduce blood lost during surgery is the use of tranexamic acid. Tranexamic acid can be administered several different ways. Typically, 1 gram of tranexamic acid is

administered intravenously for an hour before surgery and then 1 gram of tranexamic acid for 6 to 12 hours after surgery, depending on the amount of blood loss [12]. It has been shown that the use of tranexamic acid before and after surgery reduced intraoperative blood loss by 126 ml, postoperative blood loss by 215 ml and total blood loss by 408 ml. In each case the blood loss was reduced by less than or equal to 50% [13]. The use of tranexamic acid additionally reduced the need for a blood transfusion by 49% [6, 14]. Tranexamic acid has been shown to be administered topically in total hip and knee arthroplasty. Patients received 2 grams of tranexamic acid in 100 ml of normal saline which was then used to bathe the surgical site. The study showed that tranexamic acid had 119 ml of blood transfused compared to a saline bath which had 198 ml of blood transfused [15, 16]. The use of topical tranexamic acid reduced the amount of transfusion required which means the overall blood loss was reduced.

2.5. Treatment and prevention of infection

Due to the serious nature of a surgical site infection there are several ways to prevent an infection. The most common methods to prevent a SSI after spinal surgery are antibiotic prophylaxis and the use of vancomycin powder in the surgical site after surgery and before the closing of the surgical site [7]. To prevent an infection, 1 to 2 grams of vancomycin powder is spread into the surgical wound, and in rare cases, up to 6 grams of vancomycin powder can be used [17]. The use of vancomycin powder has been shown to bring infection rates down from 8.5% to 0% [18-21]. When vancomycin powder is used in the surgical wound, significant levels of vancomycin can be measured in the surgical drain. On the day of the surgery, up to 1457 µg/ml can be found in the drain. The day after surgery, 462 µg/ml is found and on the 2nd after surgery 270 µg/ml can be found

[22]. Most of the vancomycin is being flushed out of the wound and isn't being used to prevent the infection. The current method to prevent infection is very inefficient, wastes antibiotics and exposes the patient to high levels of antibiotics. If the infection is not prevented the wound site must be treated to eradicate the infection. The first way to treat an infection is aggressive surgical treatment such as debridement. Debridement treats the infection by surgically removing the dead tissues and the instrumentation used in the surgery [7, 23]. The surgical site is then irrigated with antibiotics until the infection is eradicated and then another surgery is needed to replace the instrumentation. In spinal fusion surgery, the instrumentation cannot be removed without a risky surgery and possibly progressing the spinal deformity being treated. The instrumentation is needed in spinal fusion to preserve the spines stability [2]. The second step to treat a SSI after spinal fusion is a course of antibiotics such as vancomycin. After an infection is found in the surgical site antibiotics are given intravenously for a mean time of 6 weeks [24, 25]. After the six weeks of intravenous antibiotics the patient will often undergo a course of oral antibiotics for mean time of 3 to 6 months until the infection is eradicated [2, 26].

2.6. Vancomycin and tranexamic acid used

Vancomycin is a glycopeptide antibiotic that was first discovered from a soil sample in Borneo in 1956 by a soil screening program at Eli Lilly [27]. Vancomycin inhibits cell wall synthesis by binding to the D-Ala-D-Ala terminus of peptidoglycan pentapeptide, which as a result, inhibits transglycosylase, preventing peptidoglycan elongation and cross-linking against Gram-positive organisms such as *S. aureus* [27, 28]. Vancomycin has a molecular mass of 1449 g/mol. It is typically thought of a last resort drug [29]. Some side effects of vancomycin are redness, swelling and hyperpigmentation.

Vancomycin is not effective when administered orally. It must be administered intravenously or directly into the wound [29].

Tranexamic acid is a synthetic derivative of lysine and is an antifibrinolytic agent that reduces blood loss. Tranexamic acid reduces blood loss by inhibiting the binding of fibrin to plasmin which stops the activation of plasminogen. Plasmin is responsible for breaking down fibrin clots [30]. By inhibiting the activation of plasminogen to plasmin, clots cannot be broken down and blood loss is reduced [14]. The molecular mass of tranexamic acid is 157.21 g/mol. Tranexamic acid is generally administered intravenously or in a solution [6].

2.7. Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid) (PLGA) is a copolymer consisting of the monomers lactic and glycolic acid. PLGA is a Food and Drug Administration (FDA) approved polymer for drug delivery use in humans. PLGA is widely used due to its biocompatibility and tailored biodegradation rate [31]. PLGA can act as a carrier for various drugs such as peptides, proteins and antibiotics. PLGA releases its drug through a network of pores. PLGA undergoes hydrolytic degradation by the cleavage of its ester linkages which are present in the backbone of PLGA. Upon degradation, the byproducts of PLGA are nontoxic and are naturally produced through normal metabolic pathways. These metabolic pathways are used to eliminate PLGAs byproducts [31]. There are many different properties that affect the degradation rate of PLGA. Some of these include crystallinity of the polymer, polymer chemistry (acid end group or aliphatic end group) and ratio of lactic to glycolic acid [31]. PLGA with an acid end group will degrade 2-4

fold faster depending whether it is in vivo or in vitro. The acid terminated PLGA degrades quicker due to its faster uptake of water [32]. An application of PLGA is the formation of microspheres for controllable drug delivery. It was shown using 70:30 PLGA and 90:10 PLGA to deliver vancomycin to the brain. The PLGA microspheres were created using a double emulsion and they were able to achieve a release of 63 days [33]. Antibiotic coated implants are a growing field in research. Implants can be coated with a thin film or layer of antibiotics to prevent or treat infection in surgical wounds [34, 35]. Other antibiotic delivery systems can include particle injections, bone cements, skin patches and antibiotic coatings on implants.

2.8. Gelatin

Gelatin is derived from natural proteins which can be derived from the skin of pigs, cattle bones and in rare instances, fish scales. Gelatin is denatured collagen which consists mainly of three amino acids glycine, proline and hydroxyproline [36]. Gelatin is biocompatible, biodegradable, non-immunogenic and a FDA approved polymer for human consumption. Gelatin is also approved by the FDA for use in some surgical products, one product is a gelatin sterile sponge which is used to prevent blood loss [37]. This makes it a very useful polymer for drug delivery and controlled release. Gelatin has a history of being used for tissue engineering scaffolds, hydrogels, microspheres and drug delivery matrices [38]. Gelatin is insoluble at room temperature, but soluble at body temperature. Gelatin can be crosslinked by various compounds. One of these compounds is glutaraldehyde which used to form new bonds and slow down the degradation rate through crosslinking. Schiff base linkages are formed in the gelatin, this occurs through

the formation of imines between ϵ - amino groups from the gelatin and the glutaraldehyde [39, 40].

2.9. Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a derivative of cellulose which, is a polysaccharide that is naturally water soluble [41]. CMC is derived from cellulose through an alkali-catalyzed reaction. CMC differs from cellulose because it is soluble and chemically active. CMC is a FDA approved food additive and is in many nonfood products such as toothpastes and detergents. CMC is mainly used in the food industry as a viscosity modifier or thickener [42]. CMC is additionally approved for use in various surgical products, one product is an adhesion barrier which prevents undesired contact between tissues [43]. In current research CMC is being used in many various different ways, a few ways CMC is being used are to create CMC microparticles for microfluidics and as a viscous solution for the injection of gelatin particles [44, 45].

2.10. Spray systems

Topical antimicrobials have been in use since the dawn of civilization. Some examples of these include various molds and plant extracts to treat burns or infections [46]. It wasn't until after World War I in 1925 that the first spray system was designed. The topical spray system was first used at Henry Ford Hospital to treat burn wounds. The spray system used a 2.5% tannic acid aqueous solution as the fluid medium. This aqueous solution was directly sprayed onto the burn wound [47]. The designer, Davidson, hypothesized that the spray system would reduce pain and keep the wound site cleaner due to the removal of physically interaction with the wound to apply drugs [48]. Topical

sprays allow the antibiotics or antimicrobials to be delivered to a wound site without physically touching the wound. There are many different sprays system in use today that are administered orally or nasally such as Zicam, Nasacort and Albuterol.

2.11. Significance

The goal of this project was to improve the outcomes of spinal surgery today. By combining tranexamic acid and vancomycin into a single delivery spray system, two different treatments can be performed simultaneously. This will help reduce blood loss and infections in the surgical wound, which will reduce patient costs and many other issues. The overall goal was to further improve success of procedures by developing spray system to uniformly deliver agents for reducing blood loss and preventing or treating infection. This was achieved with the short term delivery of tranexamic acid, the intermediate and longer-term release of vancomycin.

METHODS AND MATERIALS

3.1. Poly(lactic-co-glycolic acid) microspheres

PLGA microspheres were prepared using a double-emulsion process ($W_1/O/W_2$) [49]. Vancomycin (GOLDBIO; St. Louis, MO) was loaded into phosphate-buffered saline (PBS) with a pH of 7.4 at 150 mg/ml as the W_1 phase. Tranexamic acid (Acros Organics; Bridgewater, New Jersey) was loaded into PBS at 150 mg/ml or 75 mg/ml as the W_1 phase. One g of PLGA (75:25 L:G, ester-terminated, inherent viscosity 0.55-0.75 dL/g; Durect; Cupertino, California) was dissolved in 15 mL of dichloromethane (DCM) as the oil phase. The W_2 phase consisted of 1.75 g of methylcellulose and 25 g NaCl dissolved in diH₂O. The W_1 phase (1 mL) was added to the oil phase and sonicated three times for 20 seconds each to prevent excessive heating. 300 mL of the W_2 phase was homogenized at 4000 rotations per minute (RPMs) for 5 minutes, during the homogenization the W_1/O mixture was poured into the W_2 phase. The mixture was then stirred overnight to allow the DCM to evaporate. After being stirred overnight the solution was centrifuged down to remove the water phases and to rinse the microspheres. The collected microspheres were then frozen and lyophilized for 48 hours. The lyophilized microspheres were then weighed and the loading was determined.

The loading of the vancomycin or tranexamic acid encapsulated PLGA microspheres was determined by dissolving 30 mg of microspheres in 3 mL of DCM for 2 hours. Once the PLGA was dissolved 10 mL of PBS was added to the mixture and vortexed for 1 minute. Then the mixture was centrifuge for 5 minutes at 1500 RPMs to separate the liquid and oil phase. After centrifugation for the vancomycin loaded microspheres, 300 μ L was pipetted from the liquid phase in a well plate, which was used to measure UV absorbance

on a UV plate reader. The samples were measured at 280 nm, and the absorbance values measured were compared to a standard curve for vancomycin. For the tranexamic acid microspheres 2 mL of the liquid phase were pipetted into a 6 mL glass vial, then 2 mL of a ninhydrin solution (0.2% in methanol) was added to the mixture and vortexed. Then the samples were heated in a convection oven at 85 °C for 40 minutes. After the 40 minutes, the samples were cooled to room temperature. Once cooled, the samples were measured for the visible light absorbance at 571 nm. A working standard was prepared with the samples to calculate the loading of tranexamic acid.

3.2. Gelatin microspheres

Gelatin microspheres were prepared using an adapted single-emulsion process [50]. Type A gelatin powder (bloom number of 225-325) derived from porcine skin (Sigma Aldrich; St. Louis, MO) was dissolved in deionized water at 10% (wt/vol). The gelatin was then added to 40 °C olive oil that was being homogenized at 4000 RPMs for 10 minutes. Once complete the solution was moved to a stabilized 10 °C water bath for 30 minutes while being stirred, then a 25% volume of acetone was added and stirred for 60 minutes. The mixture was centrifuged to remove the oil phase and the collected gelatin microspheres were washed with cold acetone (4 °C) three times to remove any remaining oil phase. The resulting microspheres were then crosslinked for 12 hours with a 20 mM glutaraldehyde solution. After 12 hours the crosslinked microspheres were washed with deionized water and suspended in 50 mM glycine for 2 hours. The solution was suspended in glycine to inactivate remaining glutaraldehyde. The resulting microspheres were then washed in acetone and lyophilized for 24 hours.

Freeze-dried microspheres were loaded with vancomycin (150 mg/ml) or tranexamic acid (75 mg/ml) by absorbing 800 μ L for every 200 mg of microspheres. The microspheres were stored at room temperature for 3 hours and then freeze dried for 24 hours. Loaded, dried microspheres were then ground with mortar and pestle and sieved to ensure particles were below 150 μ m in diameter.

3.3. Drug analysis

3.3.1. Vancomycin

Vancomycin concentrations were determined by placing 300 μ l of the desired sample in a UV-grade 96 well plate (UV Star, VWR Scientific) and measuring absorbance at 280 nm using a Biotek Powerwave HT plate reader. Sample absorbances were compared against vancomycin standards.

3.3.2. Tranexamic Acid

To analyze the tranexamic acid content 2 mL of the desired TXA solution were pipetted into a 6 mL glass vial, then 2 mL of a ninhydrin solution (0.2% in methanol) was added to the mixture and vortexed. Then the samples were heated in a convection oven at 85 °C for 40 minutes. After the 40 minutes the samples were cooled to room temperature. Once cooled, the samples were measure for the visible light absorbance at 571 nm. A working standard was prepared with the samples to calculate the concentration of TXA in the desired solution.

3.4. Tranexamic acid spray solution

The spray solution was prepared by dissolving the tranexamic acid in PBS with a pH of 7.4 or a 1% carboxymethyl cellulose (CMC) (Sigma Aldrich; St. Louis, MO) solution at

30 mg per 1 ml of liquid. The CMC solution was made by using high viscosity carboxymethylcellulose sodium salt powder and deionized water. The 1% CMC solution was shaken at 40°C until all the powder was dissolved.

3.5. Release studies

The release of vancomycin from PLGA was studied by immersing loaded PLGA microspheres in PBS for an extended period. For the vancomycin release, 90 mg of loaded microspheres were placed into a laboratory tube with 6 mL of PBS. The samples were shaken horizontally at 86 RPMs at a constant temperature of 37 °C. The samples were centrifuged down and supernatants were collected every day for the first 3 days and every 3 days after that, for 42 days. The vancomycin concentration was determined by using UV absorbance at 280 nm.

To study the release of vancomycin from gelatin microspheres or the release of tranexamic acid from PBS or 1% CMC, samples were placed in dialysis tubing (Spectrum Laboratories). To test the release of vancomycin from gelatin, dialysis tubing (8000 Da cutoff) was loaded with 75 mg of loaded gelatin microspheres and 1.5 ml of PBS. To study the release of tranexamic acid from PBS or 1% CMC, dialysis tubing (1000 Da cutoff) was loaded with 1.5 ml of loaded PBS or 1% CMC solution (30 mg/ml). The concentration of 30 mg/ml was chosen based on literature where the saline concentration of tranexamic acid was 3 g per 100 ml [51]. The dialysis tubing was closed with weighted clips on each end. The samples were then placed in 40 ml of PBS shaking at 86 RPM at 37°C. Supernatants were collected every 2 hours for the first 12 hours, and

then everyday consecutively for 10 days. The media was changed and refreshed for each supernatant collection.

3.6. Spray gun

The water based spray bottle that was used for this research was purchased at Sam's Club and was used to deliver the microsphere solution to a target area. The spray gun was modified in several different ways. First the bottle connection was modified to fit a 50 ml centrifuge tube. This was modified by drilling a hole into the center of a tube lid and hot gluing the lid to the existing spray bottle connection. The intake tubing was then fed through the hole in the lid, to allow for fluid intake. This was done to allow a standard 50 ml centrifuge tube to be directly screwed onto the spray nozzle. Secondly, the stock intake tubing was replaced with a different tubing that had a smaller interior diameter. The stock tubing interior diameter was approximately 4.5 mm and the replacement tubing had an approximate interior diameter of 1.58 mm. The tubing was modified to allow for a smaller volume of flow which allowed more fluid to be sprayed due to the nozzle being pressure based.

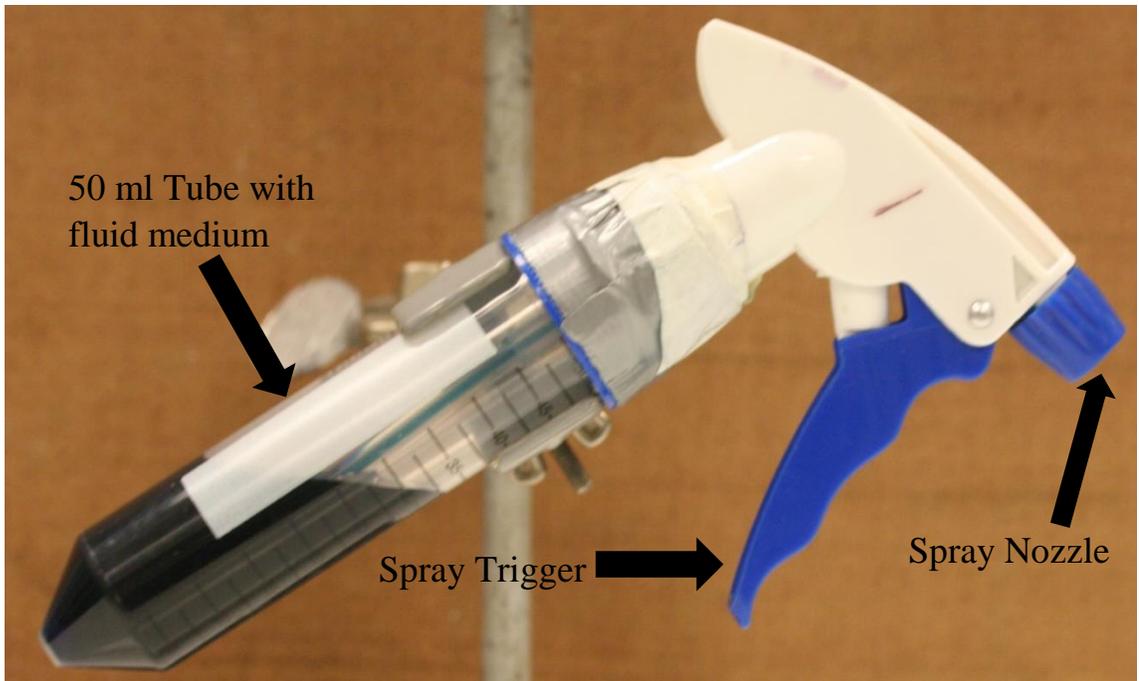


Figure 3.1: Spray gun with 50 ml tube.

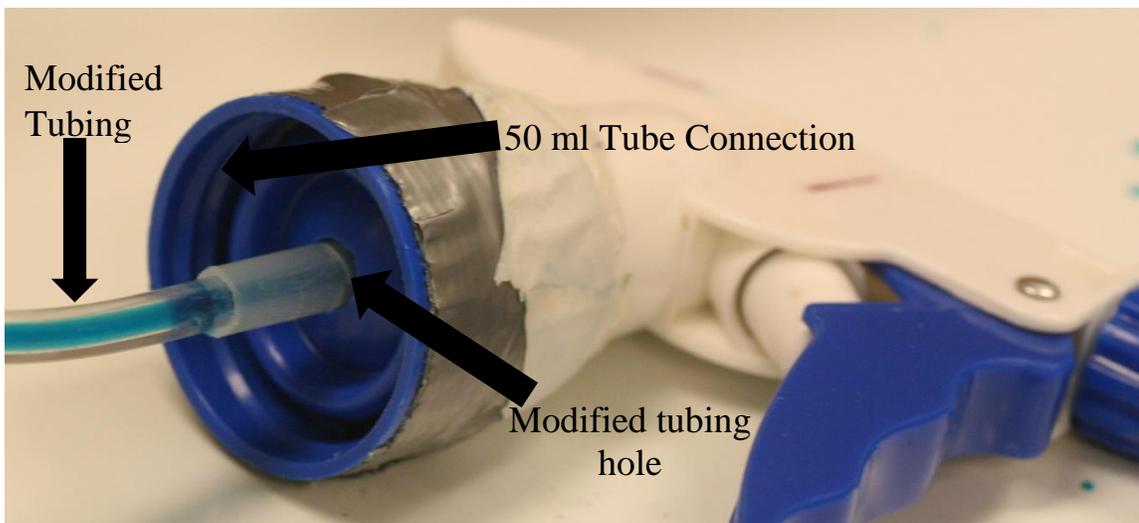


Figure 3.2: Modified tubing and 50 ml tube connection.

3.7. Spray gun experiments

3.7.1. Fluid medium

The spray solution was comprised of two components, the fluid medium and the particles. The fluid medium can either be CMC or PBS (pH 7.4). The percentage of CMC tested was between 0.25% and 3%. A test was run to determine if the fluid medium could

be sprayed or not. The particles were 20 mM crosslinked gelatin microspheres or 75:25 PLGA microspheres. The particles were immersed into the fluid medium at 50 mg/ml.

3.7.2. Volume per spray

The volume of fluid per spray was measured using PBS and 1% CMC. The nozzle of the spray gun was placed into the opening of a graduated cylinder and the trigger was pulled for a set number of sprays. The fluid sprayed was then measured using the graduated cylinder and recorded.

3.7.3. Area coverage

The area coverage of the spray system was tested by mounting the spray gun into a tube holder on a ring stand. The center of the nozzle was placed 8.5 cm above the test surface and at angle of 45°. These parameters were chosen based on consultation with Dr. Todd Milbrandt (Department of Orthopedic Surgery, Mayo Clinic) and approximate measurements based on possible use in surgery. The test area was a hydrophobic white board to allow for the greatest contrast between particles and spray solution. The spray gun was loaded with stained PLGA or gelatin microspheres spray solution and primed before testing. The system was then sprayed 5 times with pictures taken for area coverage analysis after each spray. The spray set up is shown in Figure 3.4 and Figure 3.5 below.

Federal food, drug and cosmetic (FD&C) blue #1 food dye was encapsulated in PLGA microspheres to achieve a greater amount of contrast to image and analyze the area coverage of the spray patterns. The brilliant blue food dye was encapsulated using double emulsion. It was loaded at 2 ml per 1 gram of PLGA. After microsphere formation the particles were washed 3 times using a vortex and centrifuge to remove any loose FD&C blue #1.

One gram of gelatin microspheres was stained with a Bicinchoninic Acid Assay (BCA assay) (Pierce; Rockford, Illinois). Using an adaptation of the manufacturer's protocol, 100 μ L of reagent B were mixed with 5 mL of reagent A and then added drop-wise to the gelatin microspheres. The staining resulted in the microspheres being stained purple from the BCA assay.

To dye PBS, 0.25% CMC, 0.50% CMC, 0.75% CMC and 1% CMC blue, 250 μ l of FD&C blue #1 was substituted for 250 μ l of PBS or DIH₂O. The mixtures were then heated at 60°C overnight to allow the mixture to go into solution.

To analyze the spray area coverage images several different steps were taken. First the images were enhanced to increase the amount of contrast between the spray solution and the background. Windows Live Photo Gallery was first used to automatically enhance the exposure and contrast of the images to lighten up the spray patterns. The enhanced photos were then opened in GNU Image Manipulation Program (GIMP) to crop the images and threshold them. Each image had a different level of threshold due to the subtle changes in background lighting, each image was threshold to match the original image but in black and white. Once each image was processed in GIMP they were used in ImageJ to measure the area coverage. In imageJ a scale was set using a scale bar from the original images and then the area coverage was analyzed using Analyze particles. Analyze particles has several different parameters to adjust. The size was left from zero to infinity to account for possible liquid pooling on the hydrophobic surface. Circularity, which is the roundness of the particles was left from zero to one to account for irregular shapes. The bare outlines setting was chosen to show what was measured. The particles on the

edges were not excluded. Analyze particle works by scanning across the image and once it encounters a particle it outlines it and then measures the perimeter and area of the particle using pixels. The area is converted from pixels to centimeters by using the preset scale. Analyze particles measures the total area that particles occupy in the image producing the area coverage per spray. A diagram of the image analysis is shown in Figure 3.6 below.



Figure 3.3: Area coverage spray testing set up post spray (side view).

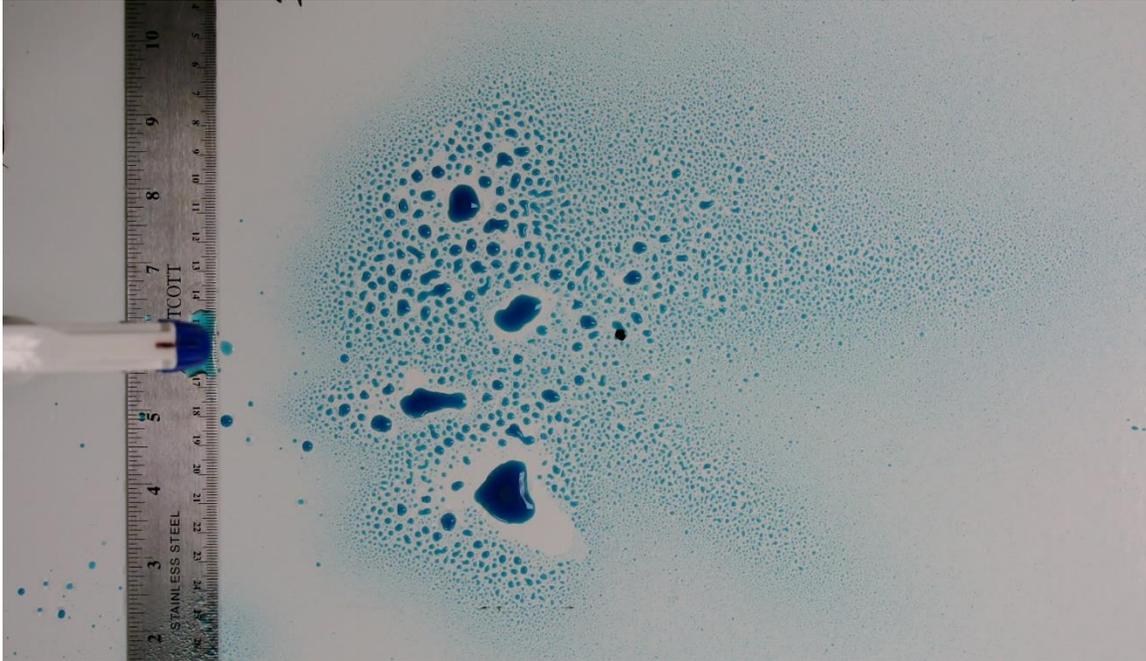


Figure 3.4: Area coverage spray testing set up post spray (bird's eye view).

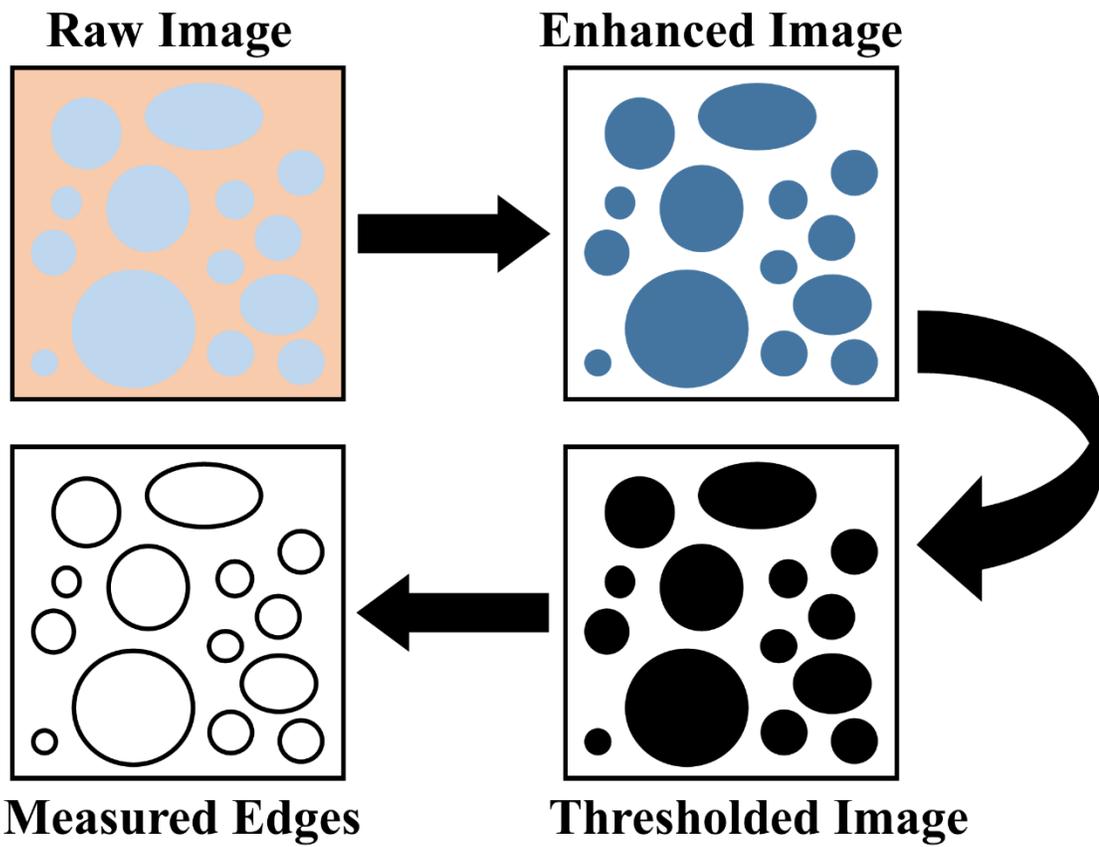


Figure 3.5: Image Analysis Diagram.

3.7.4. Mass of microspheres per spray

The mass of particles per spray was tested for both gelatin and PLGA microspheres. The empty mass of 15 mL centrifuge tubes was recorded and the spray gun was loaded with either gelatin or PLGA particles at 50 mg/ml in PBS (pH 7.4). Then particles were sprayed into the empty tubes for 1 through 5 sprays. The tubes were then frozen, lyophilized for 48 hours, and the final mass recorded. The mass of microspheres was calculated by subtracting the final mass from the initial mass of the tubes.

3.8. Drip test

The viscosity of PBS, 0.25%, 0.50%, 0.75%, 1%, 2% and 3% CMC was tested. A grid was drawn onto a hydrophobic white board to allow for easier image capture with a starting dot at the same vertical distance spaced 5 cm horizontally. To test the viscosity of the different fluids, 150 μ l of each was pipetted onto a separate starting dot. The board was then raised to an incline of 30 degrees and held there for 10 minutes. Pictures were taken every 2 minutes to document the distance traveled by each fluid. The pictures were then analyzed in ImageJ to measure how far each fluid traveled at that time point. In ImageJ the scale was set for each picture based on the grid in the pictures then using the measure tool the distance of each drip was determined and recorded. The set up for the drip test is shown in Figure 3.7 below.

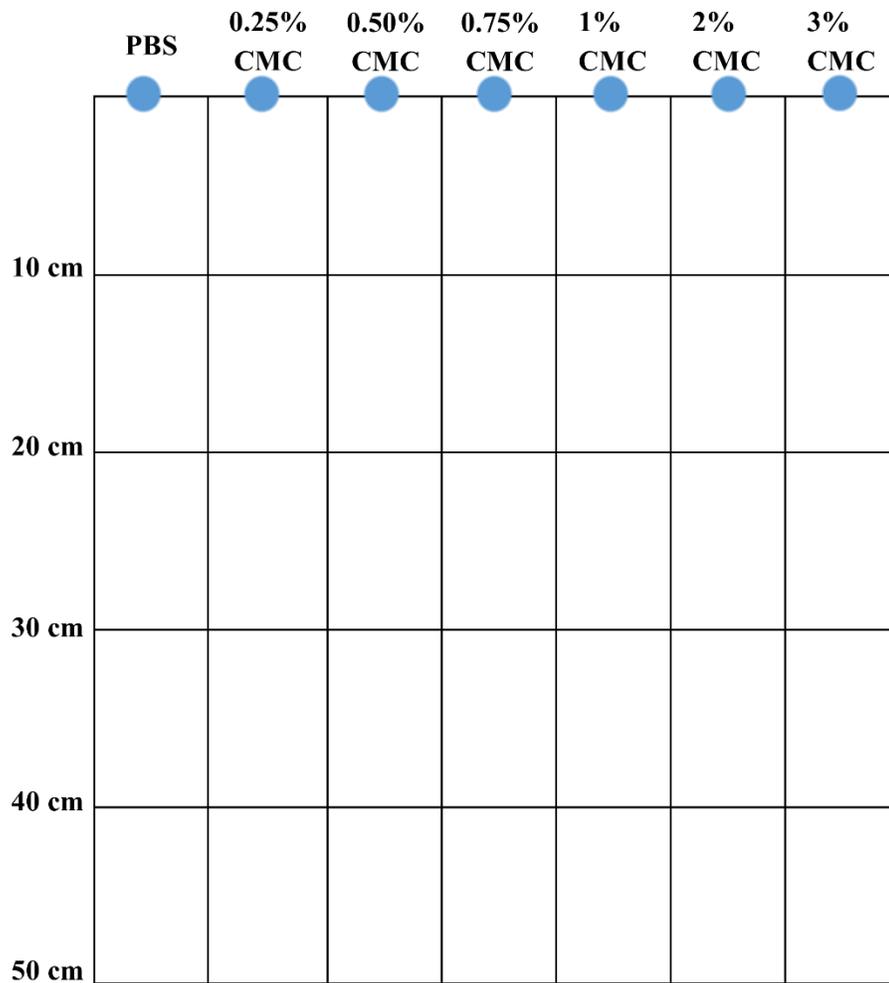


Figure 3.6: Representation of Drip Test Set Up.

3.9. Statistics

A fifth order polynomial and two-way analysis of variance (ANOVA) were run using Prism GraphPad to statistically analyze the results. Turkey's and Bonferonni's test was used for the two-way ANOVA. Extra sum-of-the-squares F test was used for the fifth order polynomial which determines statistical analysis by fitting the data with a fifth order polynomial and determining if the same polynomial curve fits each data set equally. Statistical significance was determined at p values less than 0.05.

RESULTS

4.1. Release studies and loadings

4.1.1. Vancomycin encapsulated PLGA microspheres

The release profile of vancomycin from 75:25 PLGA is shown below in Figure 4.1 part A. The vancomycin was loaded in at 5%. There was a high initial burst of vancomycin from the microspheres seen in the first 4 days which was followed by a gradual decrease in the release of vancomycin. The vancomycin was released for 42 days while staying above 6. The cumulative release of vancomycin is shown in Figure 4.1 part B.

Approximately 5.25 mg of vancomycin were released during the 42 days which was 69.7% of the total drug loaded.

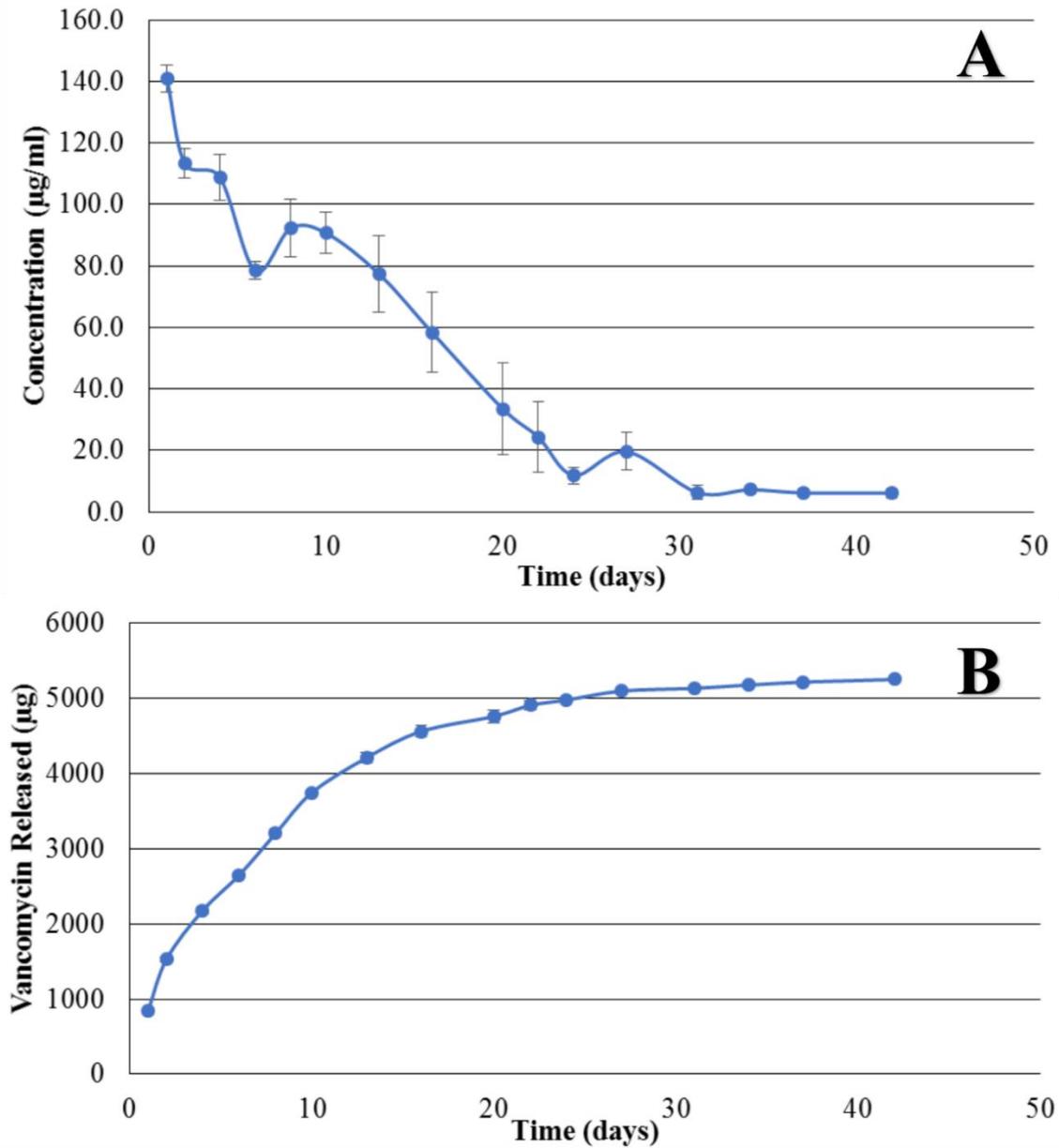


Figure 4.1: (A) Instantaneous and (B) cumulative profiles showing release of vancomycin from 75:25 PLGA. Data are mean \pm standard deviation ($n = 3$). The error bars are shown on Figure 4.1 part B but are hidden by the data points. The standard deviation ranged as high as $\pm 89 \mu\text{g}$ and low as $\pm 7 \mu\text{g}$.

4.1.2. Vancomycin loaded in 20 mM crosslinked gelatin microspheres

The release profile of vancomycin adsorbed and absorbed onto 20 mM crosslinked is shown in Figure 4.2 part A. There was a linear release of vancomycin for 48 hours with a gradual decrease in the release after 2 days until ending at day 7. The cumulative release of vancomycin from gelatin can be found in Figure 4.2 part B. The total amount of vancomycin released was approximately 20 mg.

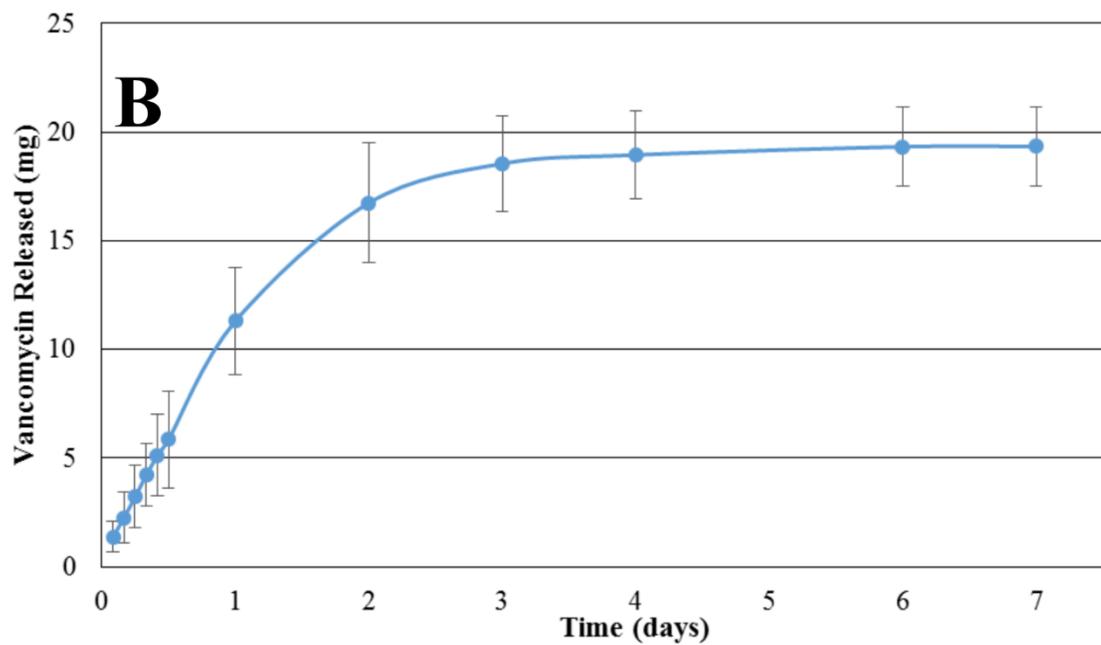
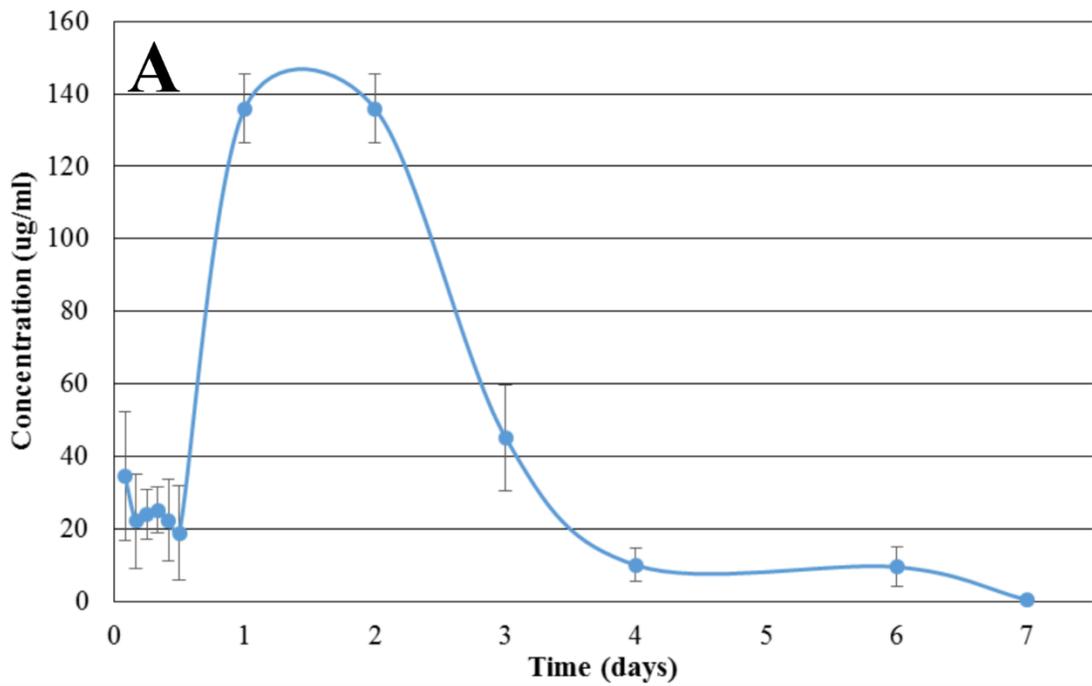


Figure 4.2: (A) Instantaneous and (B) cumulative profiles showing release of vancomycin from 20mM crosslinked gelatin. Data are mean \pm standard deviation (n = 3).

4.1.3. Tranexamic acid loading in PLGA

In 75:25 PLGA a loading of 0.62% was achieved which had a release length of 1 day (not shown).

4.1.4. Tranexamic acid release from gelatin microspheres

The release profile for tranexamic acid from 20 mM crosslinked gelatin in PBS and 1% CMC is shown in Figure 4.3 part A. Both formulations had an initial burst of tranexamic acid and the release decreased until concluding at 12 hours. A fifth order polynomial was run to determine the statistical difference between the release of tranexamic acid from gelatin in PBS and CMC. The statistical analysis found that there was no significant difference between the data. The cumulative release of tranexamic acid from gelatin in PBS and 1% CMC is shown in Figure 4.3 part B. Approximately 10 and 16 mg of TXA was released for PBS and CMC.

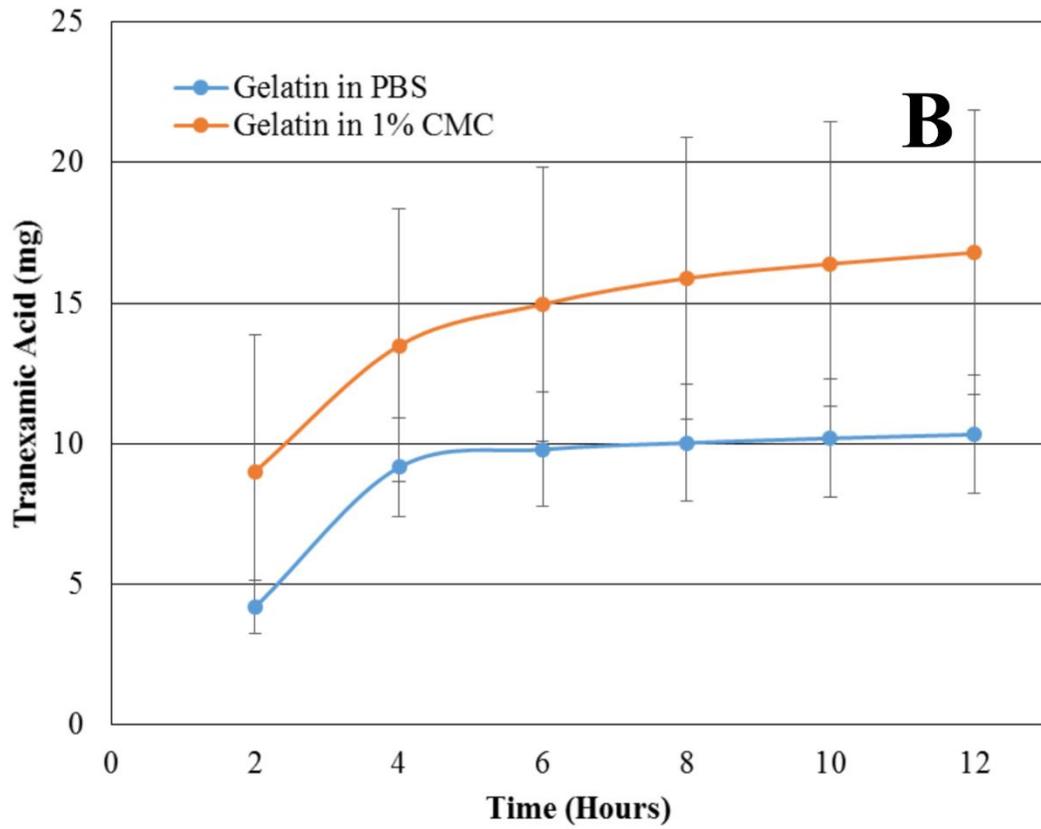
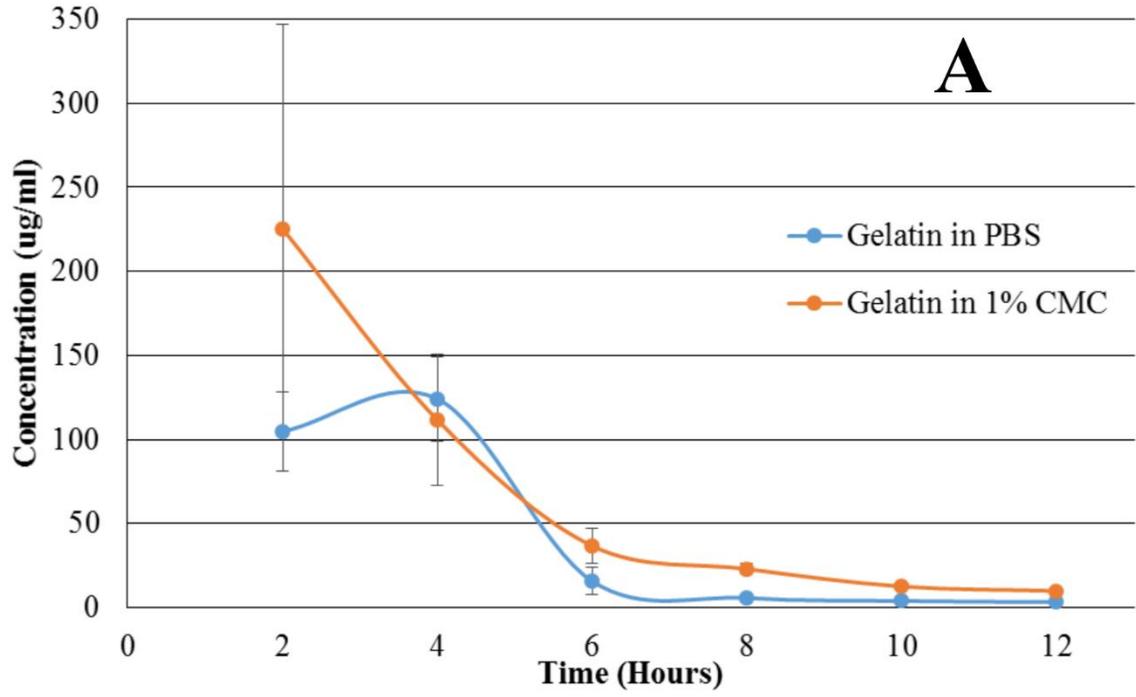


Figure 4.3: (A) Instantaneous and (B) cumulative profiles showing release of tranexamic acid from 20mM crosslinked gelatin in PBS and 1% CMC. Data are mean \pm standard deviation (n = 3).

4.1.5. Tranexamic release profile from PBS and 1% CMC

The release profile of tranexamic acid from PBS and 1% CMC is shown in Figure 4.4 part A. There was an initial burst of tranexamic acid in the first hour of the release and it continues to slowly release until the PBS and CMC finished releasing the tranexamic acid in 7 hours. A fifth order polynomial was run to analyze the statistical difference between the release of TXA from PBS and CMC. No statistical difference was found. The cumulative mass release is shown in Figure 4.4 part B. Approximately 25 mg of tranexamic acid was released in PBS, and 33 mg of tranexamic acid was released in CMC.

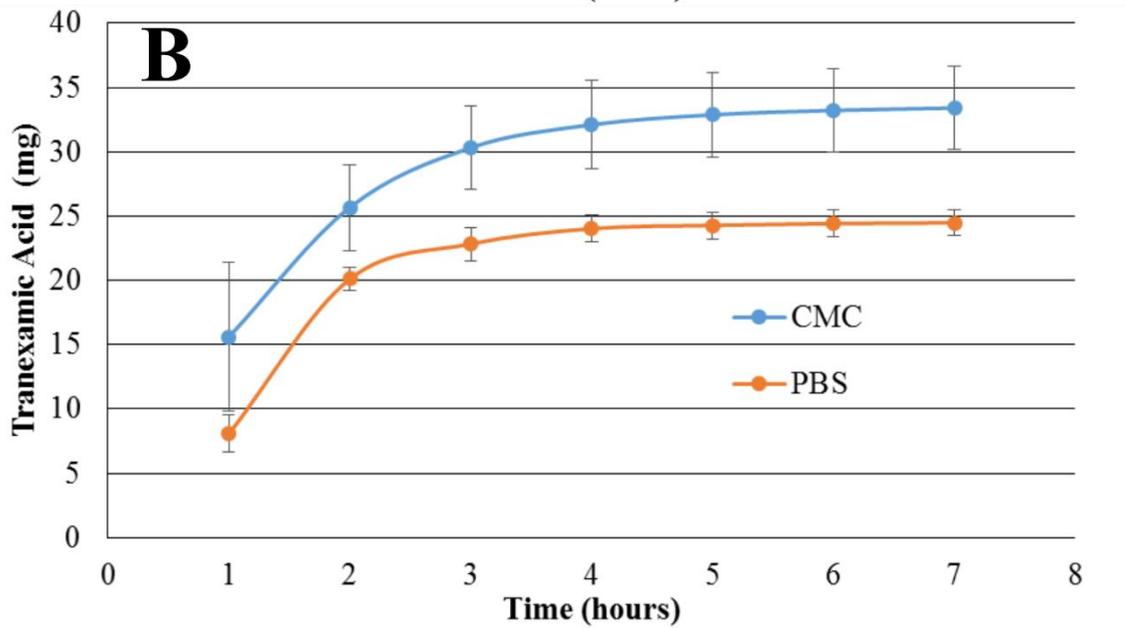
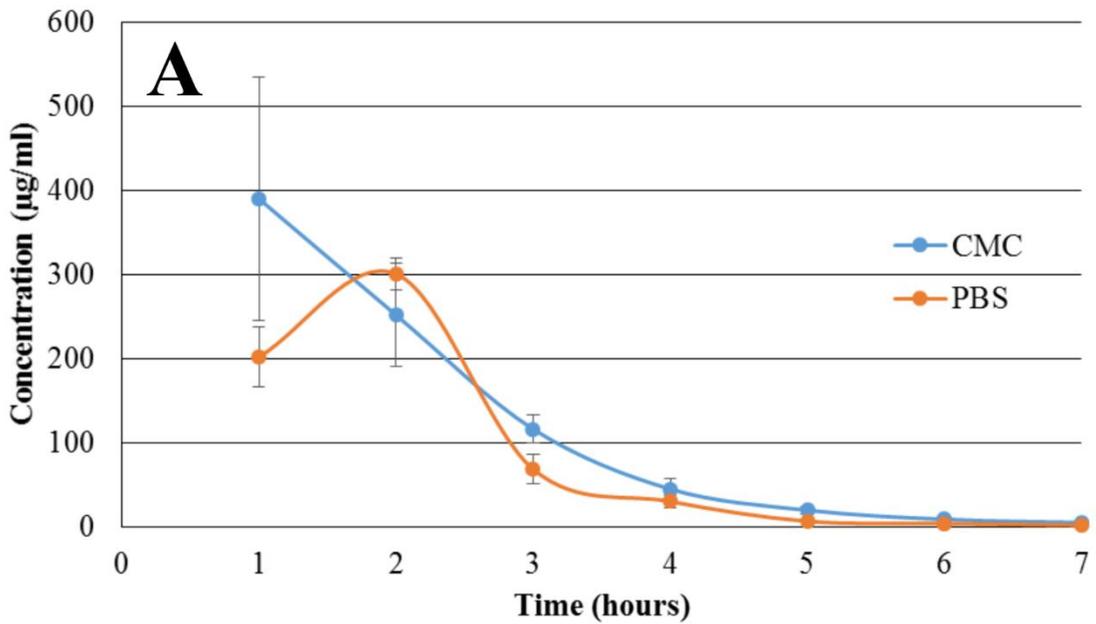


Figure 4.4: (A) Instantaneous and (B) cumulative profiles showing release of tranexamic acid from 20mM crosslinked gelatin in PBS and 1% CMC. Data are mean \pm standard deviation ($n = 3$).

4.2. Spray solution

The spray solution was tested with several different types of fluid media based on increasing percentages of CMC. All the fluid mediums were able to be sprayed when set

on a mist setting except for 2% and 3% CMC. The results of the test are shown in Table 4.1.

Table 4.1: Spray Solution Test.

Fluid Medium	PBS	0.25% CMC	0.50% CMC	0.75% CMC	1% CMC	2% CMC	3% CMC
Able to be Sprayed:	Yes	Yes	Yes	Yes	Yes	No	No

4.3. Volume per spray

The volume per spray was very consistent, the spray gun sprayed about 0.9 ml of fluid per spray regardless on the number of trigger pulls. No statistical difference was found between the two. The results for the volume per spray are shown in Figure 4.5.

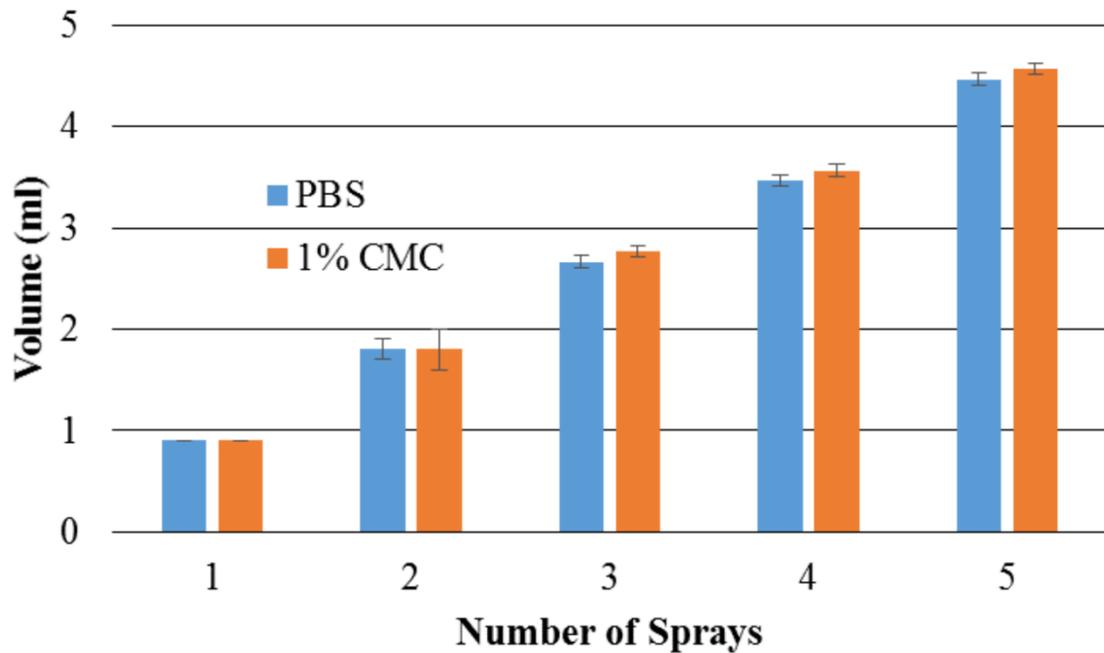


Figure 4.5: Volume per spray for the spray system. Data are mean \pm standard deviation ($n = 3$).

4.4. Area coverage

The area coverage for 7 different fluid mediums are shown in Figure 4.6. As the number of sprays increased so did the area coverage regardless of the spray medium. PBS, 0.25% CMC, Gelatin and PLGA microspheres have the greatest area coverage when suspended in PBS. Images of the 1st and 5th for area coverage of the 7 different fluid mediums is shown in Figure 4.7. The individual images were the ones used in ImageJ to measure the area coverage. For each fluid medium the area coverage increases between 1 spray and 5 sprays as more fluid medium is sprayed onto a target region which can be seen in Figure 4.7. Two way ANOVA showed statistically significant differences with respect to fluid medium, spray number, and the interaction between the fluid medium and spray number ($p < 0.0001$).

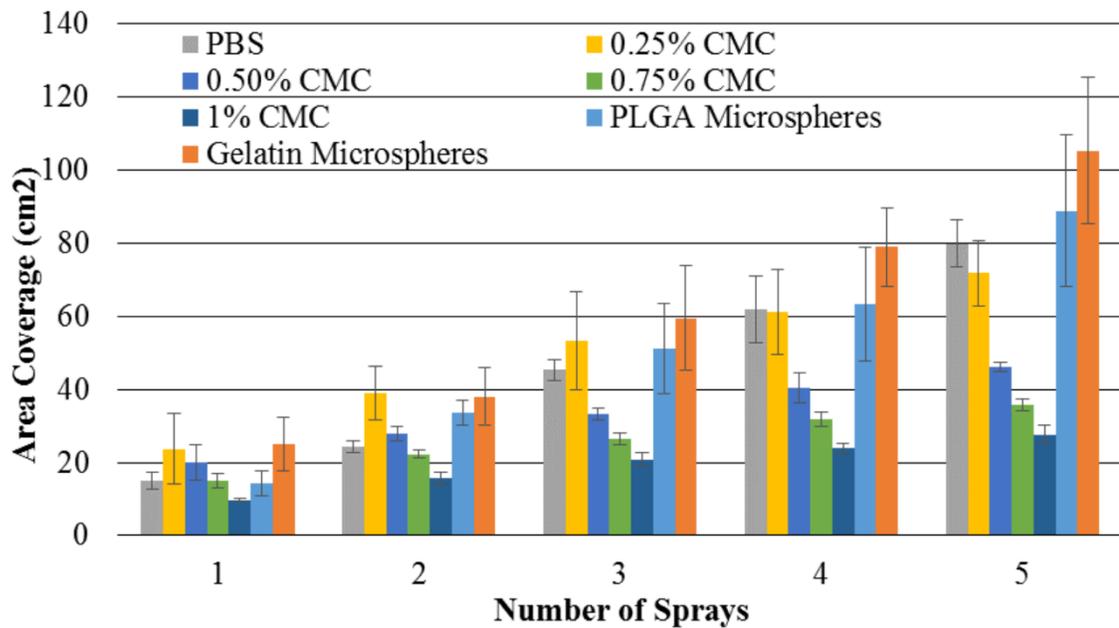


Figure 4.6: Area coverage for different numbers of sprays. Data are mean \pm standard deviation ($n = 3$)

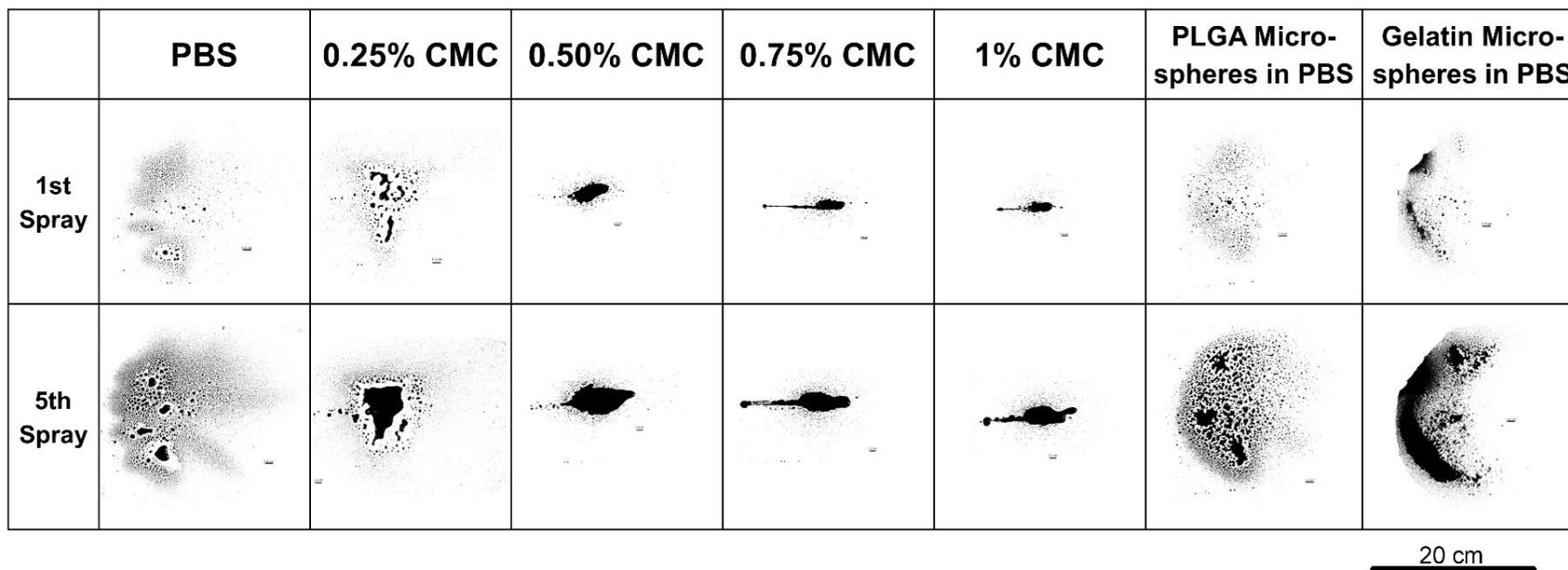


Figure 4.7: First and fifth sprays for area coverage analysis.

4.5. Mass of microspheres per spray

The mass of microspheres delivered for 1 through 5 sprays is shown in Figure 4.8 and Figure 4.9. Approximately 45 mg of microspheres should be sprayed for each pull of the trigger. This was determined from the loading of the microspheres and volume of fluid per spray. As the number of sprays increased, the mass of microspheres sprayed increased as well. No statistical difference was found between gelatin and PLGA microspheres. After running a two way ANOVA it was found that the interaction between the type of polymer sprayed and the number of sprays was statistically significant ($P = 0.011$). Two-way ANOVA also showed no statistical significance between PLGA and gelatin microspheres for the mass of microspheres sprayed.

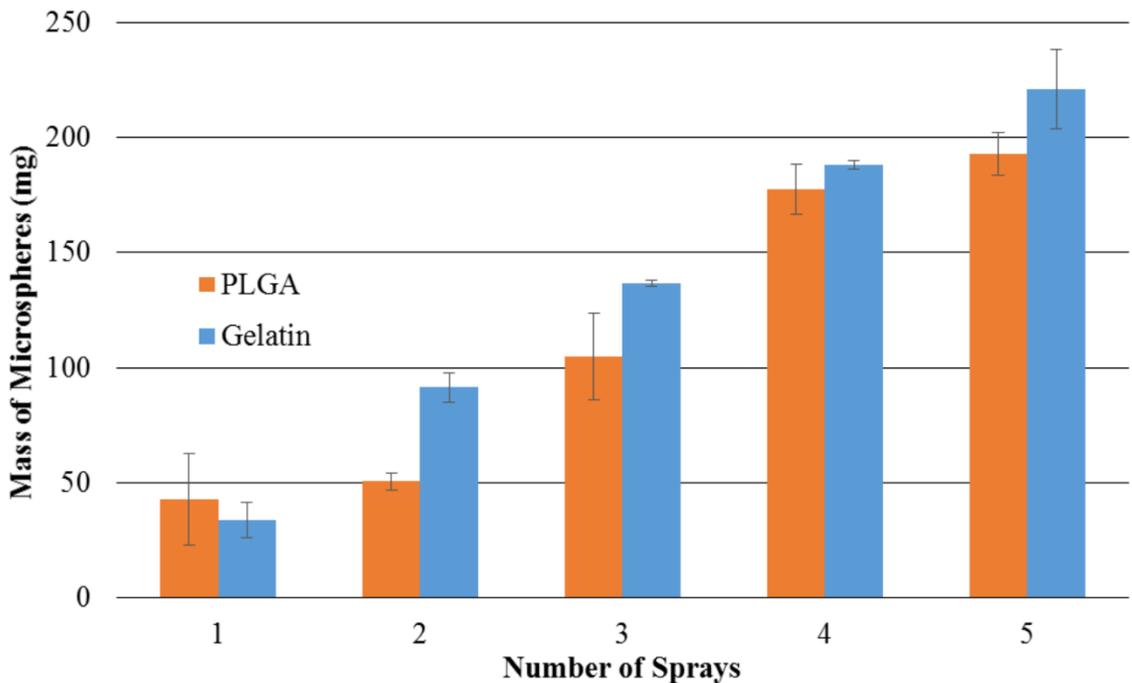


Figure 4.8: Cumulative mass of microspheres per spray for gelatin and PLGA microspheres. Data are mean \pm standard deviation ($n = 3$).

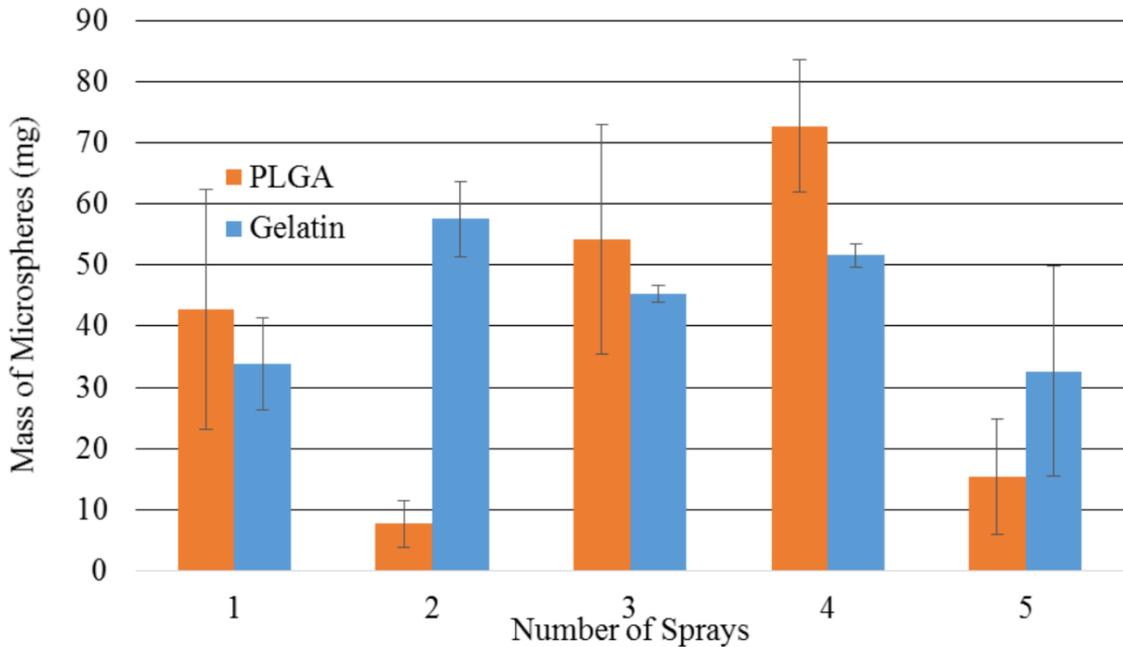


Figure 4.9: Mass of microspheres per spray for 1 through 5 sprays for gelatin and PLGA microspheres. Data are mean \pm standard deviation (n = 3).

4.6. Drip Test

For each of the fluid mediums, the distance traveled increased with time. The results from the drip test are shown Figure 4.9 and the correlating drip rates are shown in Table 4.2.

PBS reached the maximum distance that could be measured (50 cm) in under 2 minutes which is why it remained constant for the remainder of the test. PBS has the greatest drip rate of any fluid medium tested at 25.0 cm/min. The second fastest drip rate was 0.25% CMC at 4.33 cm/min. As the percentage of CMC increased, the drip rate decreased. Two-way ANOVA showed statistical significance for the fluid medium, time and the interaction between fluid medium and time ($P < 0.0001$). Significant differences were found for PBS, 0.25% CMC and 0.50% CMC when compared against all other fluid media at all time points. No significant difference was found between 0.75% CMC and 1% CMC. No significant differences were found between 1%, 2% and 3% CMC.

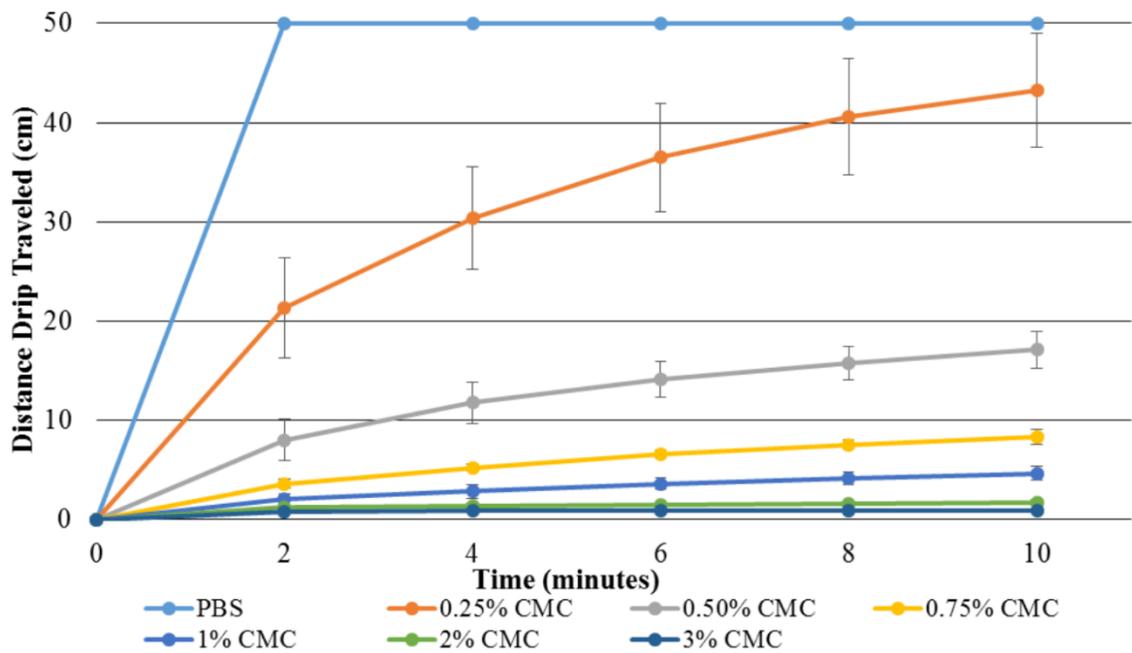


Figure 4.10: Drip test for different fluid mediums. Data are mean \pm standard deviation (n = 3).

Table 4.2: Drip rate for different fluid mediums.

Fluid Medium	Mean Drip Rate (cm/min)
PBS	25.0
0.25% CMC	4.33
0.50% CMC	1.71
0.75% CMC	0.83
1.0% CMC	0.47
2.0% CMC	0.17
3.0% CMC	0.09

DISCUSSION

Blood loss and the potential for infection remain significant complications of spinal fusion. A sprayable system was developed for localized delivery of antibacterial and antifibrinolytic agents to a target area. The antibacterial agent could be used to prevent and treat infection in the surgical site. The antifibrinolytic agent could prevent and treat blood loss after surgery and therefore reduce the risks. The spray system was created to improve upon the current techniques.

For prolonged release of vancomycin, the antibiotic was encapsulated into degradable PLGA microspheres. The concentration of vancomycin remained above 2 $\mu\text{g}/\text{ml}$ for the 42 days of the PLGA release study. This was important because it was greater than the minimum inhibitory concentration (MIC) of *S. aureus* [52]. The lowest concentration released was on day 42, which was 6.2 mg/ml. The 42 days of antibiotic release was important due to the current methods used to treat a spinal fusion surgical site infection. Current methods used to treat an infection require antibiotics intravenously for a mean time of 6 weeks. Oral antibiotics may be needed for several months after intravenous antibiotics and in some cases multiple debridements of the surgical site to fully remove the infection [7, 9, 24, 25]. Multiple debridement procedures can result in soft tissue damage and can require additional surgeries. Debridement for spinal fusion surgery involving instrumentation can be difficult or impossible to perform. If performed, there is an increased risk for pseudoarthritis [9, 24, 25]. The controlled release of the antibiotic for 6 weeks may allow the PLGA microspheres to be used to treat a postoperative spinal fusion infection.

Vancomycin released from gelatin microspheres had a sustained release of 7 days with a delay in the initial burst. The initial delay in the burst was hypothesized to be due to electrostatic interactions between the vancomycin and gelatin microspheres [53]. The 7 day mark was important due to the need to prevent infection after spinal fusion surgery. The release of vancomycin stayed well above the MIC for *S. aureus* until the 7 day mark. The current technique used to prevent infections (1 gram of vancomycin powder spread on the wound) provides 3 days of detectable vancomycin levels in the wound drain and successfully prevents infection [8, 22]. Infection rates were reduced from as high as 8.5% to 0% after application of the vancomycin powder in the surgical site [7, 54]. The controlled release of vancomycin for 7 days may allow the gelatin microspheres to prevent surgical site infection. Therefore, it could be used as an alternative prevention method.

PLGA and gelatin microspheres were each tested in a variety of ways to determine whether they could be used in the spray system. Area coverage was an important test because if the microspheres were being distributed onto the target region it means the encapsulated drugs are also being distributed over the target region. A large area coverage was also important as well. A larger area coverage would indicate that the microspheres are being distributed over a greater area of the wound site. This would allow the vancomycin that was encapsulated to work over a greater area and prevent/treat infection over a greater region. The average surgical wound size for a single spinal fusion is approximately 10 cm wide by 12 to 15 cm long [55]. For multiple spinal fusion surgery the wound size is approximately 10 cm by 28 to 30 cm long, based on consultation with Dr. Todd Milbrandt (Department of Orthopedic Surgery, Mayo Clinic). For each case this

results in the total area of the wound site to be between 120 to 150 cm² for a single fusion and between 280 to 300 cm² for multiple fusions. PLGA and gelatin microspheres had area coverages of approximately 90 and 120 cm² at five sprays, respectively. The area coverage test was static, meaning the spray system was not moved around, which resulted in a large amount of pooling of the fluid medium. It is hypothesized that if the spray system was dynamic the area coverage would increase. Nonetheless, the area coverage testing showed that the PLGA and gelatin microspheres could be used to distributed microspheres encapsulating vancomycin onto the entire surface of the wound site with more sprays.

The next test performed with the PLGA and gelatin microspheres was the mass of microspheres per spray. This was an important test because it was used to verify that microspheres were in fact being sprayed and what mass of microspheres were approximately being sprayed for each pull of the trigger. This test was also used to validate that the area coverage measured was from the microspheres and the fluid medium not just the fluid medium solely. It was expected for no statistical difference to be found between PLGA and gelatin microspheres due to equal particle density (mass/volume). There was some error in the test which resulted from the need to prime the spray gun before the first spray. Priming the spray gun means that there was no spray solution in the intake tubing during the first pull of the trigger, therefore the trigger must be pulled several times to start the uptake of the spray solution. This can skew results because the first few sprays may only have a partial or lack of spray solution expelled. This problem would be addressed in the development of the device for the market. The spray system delivered approximately 40 to 45 mg of PLGA or gelatin microspheres per

spray when properly primed. Knowing the loading of vancomycin in the microspheres and the mass of microspheres sprayed could be used to calculate the appropriate dosage of vancomycin for the patient. At this time the current dose of vancomycin most commonly used is 1 gram of vancomycin powder spread into the surgical site [8, 17, 54]. Most of the vancomycin is flushed out the wound very quickly through the wound drainage [22]. On the day of the surgery and one day after, approximately 1450 $\mu\text{g/ml}$ and 450 $\mu\text{g/ml}$, respectively, of vancomycin were found in the wound drainage [22]. Therefore, the use of 1 gram of vancomycin is very wasteful because most of the vancomycin is quickly flushed out of the wound. To properly prevent infection the concentration of vancomycin must remain higher than the MIC of the most common organism *S. aureus*. The MIC of *S. aureus* is 2 $\mu\text{g/ml}$ and the average volume of fluid loss during spinal fusion is between 750 and 1500 ml [56]. To ensure that the infection is being prevented we can aim for a concentration of 10 $\mu\text{g/ml}$ of vancomycin. This concentration was chosen to account for any possible loss of antibiotic due to wound drainage. To achieve a concentration of 10 $\mu\text{g/ml}$ we would need to spray 15 mg of antibiotic into the wound, and if the microspheres are loaded with vancomycin at 5% we would need to start with 300 mg of antibiotic. Using this method to prevent infection you can reduce the total amount of antibiotic needed by 700 mg and you can reduce the amount entering the wound site by 985 mg.

The size of the microspheres sprayed could play a large role in several different aspects of the spray system and lifespan of the particles. If the microspheres sprayed were too large they could clog the spray system which would prevent the system from working properly. Depending on the size of the microspheres they could be phagocytized by

macrophages which are present during wound healing. It has been shown that polymeric particles can be phagocytized if their diameter is between 1 and 9 μm [57]. The PLGA microspheres used in this study were found to be 40 μm or greater in diameter. The gelatin microspheres were sieved with a maximum diameter of 150 μm , but they will swell in the presence of water to increase in size. Therefore, phagocytosis of the microspheres used in a wound is unlikely due to the size of the particles used. Frustrated phagocytosis is likely to occur, however. This occurs when the substrate is too large to be phagocytized by macrophages. In this case, the macrophages will fuse together to form a foreign body giant cell and start to release superoxides and free radicals, which can damage the microspheres as well as surrounding tissues [58].

At this time no other spray system is being used to treat/prevent surgical site infections in spinal fusions. There are several different systems that can be used to deliver antibiotics. These systems can be injections, inhalable, bone cements or topical sprays. Injections can have antibiotics encapsulated in particles to be delivered to a localized site for drug delivery [44]. Inhalable drugs such as steroids are often used to treat lung diseases [59]. Another controlled release system that has been found was bone cement with antibiotics mixed directly into poly (methyl methacrylate) which is not relevant for this surgery [60]. Lastly spray systems are being used to treat burn wounds. Currently an aerosol haruan spray is being used on burns. It has been found that it reduces infection and promotes rapid healing. The haruan is in a saline solution and directly sprayed onto the burn site [61].

Several different experiments were run to test the spray system and the possible fluid media for the spray system. One experiment run was a drip test on seven different fluid media. The drip test was run to determine the viscosity of the fluid media. As stated in the results as the percentage of CMC increased the viscosity increased as well. In a surgical site there is major blood loss and poor fluid retention due to the high volume of blood loss. The average blood loss in spinal fusion surgery ranges between 750 and 1500 ml [5, 56]. There have been some reported cases of blood loss as high as 2839 ml in a spinal fusion surgery [5]. The hypothesis of the drip test was that if a higher viscosity fluid was sprayed onto the wound it would adhere to the surgical wound more and would not be lost as quickly. If the fluid media had a higher viscosity it would help prevent or reduce the time it takes for the fluid sprayed to leave the surgical site. This would allow any of the drugs sprayed to stay in the wound longer and provide them with more time to function.

The sprayability experiment tested seven different fluid media in increasing viscosity. The only two fluid media that could not be sprayed were 2.0% CMC and 3.0% CMC. The system became clogged due to the viscosity of the fluid medium. When the system became clogged, the trigger was no longer able to be pressed. This test was used to determine which fluid media to test area coverage and the drip test on.

The next test was run on the spray system and the fluid media. The test run was volume per spray. This was an important experiment because it proved that the volume per spray is roughly 0.9 ml per spray for either fluid media. To calculate the dosage for the anti-fibrinolytic agent the user only needs the loading of TXA in the fluid media and the

volume per spray. Therefore, if the loading of the tranexamic acid is 30 mg/ml and the user sprays 5 times the total amount of tranexamic acid sprayed is 135 mg.

The last experiment run was the area coverage testing of the different fluid media. Much like the area coverage testing of the microspheres, this experiment was run to verify that the different fluid mediums were being distributed onto the target region. The experiment was also run to determine the trend of area coverage as the percentage of CMC increases. Due to no significant difference being found between PBS and 0.25% CMC, the two fluids could be used interchangeably without compromising area coverage. The 0.25% CMC could be used to distribute the microspheres or anti-fibrinolytic to a large region of the target area. The 0.25% CMC would also provide increased viscosity without a reduction in area coverage when compared to PBS. All higher percentages of CMC had increased viscosity but with a reduction in area coverage. When sprayed, PBS and 0.25% CMC came out in a mist, while 0.5%, 0.75% and 1% CMC came out in a stream on the same setting. The fluid media was too viscous to spray in a mist.

Tranexamic acid was successfully loaded into 75:25 PLGA with a loading of 0.6%. When a release study was performed on the 75:25 PLGA the tranexamic acid was released in 24 hours. This release length is favorable for tranexamic acid because postoperatively it can be administered between 6 and 12 hours [5, 12]. The release length was unfavorable for PLGA due to the degradation length of 75:25 PLGA. The drug would be released in 24 hours and the PLGA would remain in the wound site for several more weeks, which could possibly cause inflammation [31, 62].

Tranexamic acid was adsorbed and absorbed onto gelatin microspheres after trying PLGA microspheres. A release study was performed to determine the duration of tranexamic acid release. The release profile achieved had 12 hours of release in PBS and 1% CMC. The fluid medium did not change the release of tranexamic acid. This was favorable because a higher viscosity solution could be used without altering the release profile of the drug. Once again though the release length was typical for tranexamic acid but unfavorable for gelatin microspheres. The degradation length of gelatin was much longer which could cause inflammation in the wound site [63].

Based on the normal clinical dosing length of tranexamic acid a release study was run on tranexamic acid dissolved into PBS and 1% CMC. No polymer was used. The resulting release profile was 6 hours of tranexamic release from both the 1% CMC and PBS. This indicates that the release length of tranexamic acid was not dependent on the fluid medium. The release profile was favorable for tranexamic acid because long term dosing is not required due to the uses of tranexamic acid [6, 15, 16, 30]. It has been shown that tranexamic acid administered up to 2 to 6 hours after surgery will reduce blood loss for 24 hours [5, 13, 15].

Antibacterial sprays are not a novel concept. An antibiotic topical spray was tested for possible use in combat wounds during the Vietnam War. The spray system consisted of 100 mg of oxytetracycline hydrochloride, isopropyl myristate and a propellant. The 10 cc solution was sprayed onto the wound to prevent infection. It was shown that the infection rate was reduced when using the spray but debridement was still necessary to prevent infection, the spray system only prevented the onset of infection for a few days [64].

Another spray system was used during the 1980s to determine whether an antibacterial spray could prevent infection after an appendectomy. The spray system was a povidone-iodine dry powder aerosol spray. It was sprayed into the surgical wound and again after the closing of the wound to ensure there was a good coating of powder. No significant results were found using the povidone-iodine spray. The spray did not prevent or reduce infection rates [65]. There are also a large number of antibacterial spray systems on the market today. Some examples of these antibacterial sprays are Dermoplast, Bactine sprays and Neosporin sprays. These can all be bought at any pharmacy without a prescription.

Currently no spray systems found utilize the controlled release of antibiotics. The microsphere spray system was able to utilize the controlled release of antibiotics to prevent or treat infection. The microsphere spray system also had the combined release of tranexamic acid release from the fluid media. These combined features make the microsphere spray system a novel and practical solution to improving spinal fusion surgery recovery.

CONCLUSION

The goal of this research was to develop a better method and system to prevent and treat blood loss and infection in spinal fusion surgery. The spray system for wound coverage demonstrated delivery of antibacterial and antifibrinolytic agents to a target area through the area coverage and mass of microspheres per spray. The area coverage confirmed that particles would be distributed to a large area of the surgical site maximizing the potential of the drugs encapsulated. The use of CMC allowed the spray solution to have increased viscosity to reduce the loss of drugs. The release studies showed that vancomycin can be released for 42 or 7 days, depending on the use of PLGA or gelatin microspheres, respectively. The tranexamic acid was shown to be released from PBS or CMC by diffusion over 6 hours. The different drug solutions can be used to treat or prevent infection and blood loss in spinal fusion surgery. Animal testing is required to fully support that this system is an improvement upon the current techniques. Future work in the project could be addressing biofilms and tailoring the spray system for a wider range of applications.

REFERENCES

- [1] Rajae SS, Bae HW, Kanim LEA, Delamarter RB. Spinal Fusion in the United States Analysis of Trends From 1998 to 2008. *Spine*. 2012;37:67-76.
- [2] Messina AF, Berman DM, Ghazarian SR, Patel R, Neustadt J, Hahn G, et al. The Management and Outcome of Spinal Implant-related Infections in Pediatric Patients A Retrospective Review. *Pediatric Infectious Disease Journal*. 2014;33:720-3.
- [3] Cha CW, Deible C, Muzzonigro T, Lopez-Plaza I, Vogt M, Kang JD. Allogeneic transfusion requirements after autologous donations in posterior lumbar surgeries. *Spine*. 2002;27:99-104.
- [4] Yoshihara H, Yoneoka D. Trends in the Utilization of Blood Transfusions in Spinal Fusion in the United States From 2000 to 2009. *Spine*. 2014;39:297-303.
- [5] Elgafy H, Bransford RJ, McGuire RA, Dettori JR, Fischer D. Blood Loss in Major Spine Surgery Are There Effective Measures to Decrease Massive Hemorrhage in Major Spine Fusion Surgery? *Spine*. 2010;35:S47-S56.
- [6] Huang F, Wu D, Ma GW, Yin ZS, Wang Q. The use of tranexamic acid to reduce blood loss and transfusion in major orthopedic surgery: a meta-analysis. *Journal of Surgical Research*. 2014;186:318-27.
- [7] Gerometta A, Olaverri J, Bitan F. Infections in spinal instrumentation. *International Orthopaedics*. 2012;36:457-64.
- [8] Godil SS, Parker SL, O'Neill KR, Devin CJ, McGirt MJ. Comparative effectiveness and cost-benefit analysis of local application of vancomycin powder in posterior spinal fusion for spine trauma. *Journal of Neurosurgery-Spine*. 2013;19:331-5.
- [9] Meredith DS, Kepler CK, Huang RC, Brause BD, Boachie-Adjei O. Postoperative infections of the lumbar spine: presentation and management. *International Orthopaedics*. 2012;36:439-44.
- [10] Stone PW, Glied SA, McNair PD, Matthes N, Cohen B, Landers TF, et al. CMS Changes in Reimbursement for HAIs Setting A Research Agenda. *Medical Care*. 2010;48:433-9.
- [11] Lee GM, Hartmann CW, Graham D, Kassler W, Linn MD, Krein S, et al. Perceived impact of the Medicare policy to adjust payment for health care-associated infections. *American Journal of Infection Control*. 2012;40:314-9.
- [12] Endres S, Heinz M, Wilke A. Efficacy of tranexamic acid in reducing blood loss in posterior lumbar spine surgery for degenerative spinal stenosis with instability: a retrospective case control study. *Bmc Surgery*. 2011;11.

- [13] Zohar E, Ellis M, Ifrach N, Stern A, Sapir O, Fredman B. The postoperative blood-sparing efficacy of oral versus intravenous tranexamic acid after total knee replacement. *Anesthesia and Analgesia*. 2004;99:1679-83.
- [14] Li ZJ, Fu X, Xing D, Zhang HF, Zang JC, Ma XL. Is tranexamic acid effective and safe in spinal surgery? A meta-analysis of randomized controlled trials. *European Spine Journal*. 2013;22:1950-7.
- [15] Martin JG, Cassatt KB, Kincaid-Cinnamon KA, Westendorf DS, Garton AS, Lemke JH. Topical Administration of Tranexamic Acid in Primary Total Hip and Total Knee Arthroplasty. *Journal of Arthroplasty*. 2014;29:889-94.
- [16] Chang CH, Chang YH, Chen DW, Ueng SWN, Lee MS. Topical Tranexamic Acid Reduces Blood Loss and Transfusion Rates Associated With Primary Total Hip Arthroplasty. *Clinical Orthopaedics and Related Research*. 2014;472:1552-7.
- [17] Ghobrial GM, Thakkar V, Andrews E, Lang M, Chitale A, Oppenlander ME, et al. Intraoperative Vancomycin Use in Spinal Surgery Single Institution Experience and Microbial Trends. *Spine*. 2014;39:550-5.
- [18] Gans I, Dormans JP, Spiegel DA, Flynn JM, Sankar WN, Campbell RM, et al. Adjunctive Vancomycin Powder in Pediatric Spine Surgery is Safe. *Spine*. 2013;38:1703-7.
- [19] Tubaki VR, Rajasekaran S, Shetty AP. Effects of Using Intravenous Antibiotic Only Versus Local Intrawound Vancomycin Antibiotic Powder Application in Addition to Intravenous Antibiotics on Postoperative Infection in Spine Surgery in 907 Patients. *Spine*. 2013;38:2149-55.
- [20] Hill BW, Emohare O, Song B, Davis R, Kang MM. The use of vancomycin powder reduces surgical reoperation in posterior instrumented and noninstrumented spinal surgery. *Acta Neurochirurgica*. 2014;156:749-54.
- [21] Martin JR, Adogwa O, Brown CR, Bagley CA, Richardson WJ, Lad SP, et al. Experience With Intrawound Vancomycin Powder for Spinal Deformity Surgery. *Spine*. 2014;39:177-84.
- [22] Sweet FA, Roh M, Sliva C. Intrawound Application of Vancomycin for Prophylaxis in Instrumented Thoracolumbar Fusions Efficacy, Drug Levels, and Patient Outcomes. *Spine*. 2011;36:2084-8.
- [23] Jin WD, Wang Q, Wang ZL, Geng GQ. Complete debridement for treatment of thoracolumbar spinal tuberculosis: a clinical curative effect observation. *Spine Journal*. 2014;14:964-70.
- [24] Hegde V, Meredith D, Kepler C, Huang R. Management of Postoperative Spinal Infections. *World Journal of Orthopedics*. 2012;3:182-9.

- [25] Li Y, Glotzbecker M, Hedequist D. Surgical site infection after pediatric spinal deformity surgery. *Current Reviews in Musculoskeletal Medicine*. 2012;5:111-9.
- [26] Maruo K, Berven SH. Outcome and treatment of postoperative spine surgical site infections: predictors of treatment success and failure. *Journal of Orthopaedic Science*. 2014;19:398-404.
- [27] Dougherty T, Pucci M. *Antibiotic Discovery and Development*: Springer US; 2012.
- [28] Gardete S, Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *Journal of Clinical Investigation*. 2014;124:2836-40.
- [29] Le A, Patel S. Extravasation of Noncytotoxic Drugs: A Review of the Literature. *Annals of Pharmacotherapy*. 2014;48:870-86.
- [30] Yang BH, Li HP, Wang D, He XJ, Zhang C, Yang PL. Systematic Review and Meta-Analysis of Perioperative Intravenous Tranexamic Acid Use in Spinal Surgery. *Plos One*. 2013;8.
- [31] Gentile P, Chiono V, Carmagnola I, Hatton PV. An Overview of Poly(lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering. *International Journal of Molecular Sciences*. 2014;15:3640-59.
- [32] Tracy MA, Ward KL, Firouzabadian L, Wang Y, Dong N, Qian R, et al. Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro. *Biomaterials*. 1999;20:1057-62.
- [33] Ozalp Y, Ozdemir N, Kocagoz S, Hasirci V. Controlled release of vancomycin from biodegradable microcapsules. *Journal of Microencapsulation*. 2001;18:89-110.
- [34] Ungureanu C, Popescu S, Purcel G, Tofan V, Popescu M, Salageanu A, et al. Improved antibacterial behavior of titanium surface with torularhodin-polypyrrole film. *Materials Science & Engineering C-Materials for Biological Applications*. 2014;42:726-33.
- [35] Zhang BGX, Myers DE, Wallace GG, Brandt M, Choong PFM. Bioactive Coatings for Orthopaedic Implants-Recent Trends in Development of Implant Coatings. *International Journal of Molecular Sciences*. 2014;15:11878-921.
- [36] Phadke KV, Manjeshwar LS, Aminabhavi TM. Microspheres of Gelatin and Poly(ethylene glycol) Coated with Ethyl Cellulose for Controlled Release of Metronidazole. *Industrial & Engineering Chemistry Research*. 2014;53:6575-84.
- [37] Cui LL, Chen PP, Tan ZQ, Li WJ, Dong ZY. Hemostatic gelatin sponge is a superior matrix to matrigel for establishment of LNCaP human prostate cancer in nude mice. *Prostate*. 2012;72:1669-77.

- [38] Adhirajan N, Thanavel R, Naveen N, Uma TS, Babu M. Functionally modified gelatin microspheres as a growth factor's delivery system: development and characterization. *Polymer Bulletin*. 2014;71:1015-30.
- [39] Phadke KV, Manjeshwar LS, Aminabhavi TM. Biodegradable polymeric microspheres of gelatin and carboxymethyl guar gum for controlled release of theophylline. *Polymer Bulletin*. 2014;71:1625-43.
- [40] Stancu IC. Gelatin hydrogels with PAMAM nanostructured surface and high density surface-localized amino groups. *Reactive & Functional Polymers*. 2010;70:314-24.
- [41] Liu J, Wu J, Zhang H. Research development on the stabilization of acidified milk drink induced by sodium carboxymethyl cellulose. *China Dairy Industry*. 2012;40:38-41, 58.
- [42] An YP, Cui B, Wang YT, Jin WP, Geng XP, Yan XX, et al. Functional properties of ovalbumin glycosylated with carboxymethyl cellulose of different substitution degree. *Food Hydrocolloids*. 2014;40:1-8.
- [43] Salum MR, Lam DTY, Wexner SD, Pikarsky A, Baig MK, Weiss EG, et al. Does limited placement of bioresorbable membrane of modified sodium hyaluronate and carboxymethylcellulose (Septrafilm (R)) have possible short-term beneficial impact? *Diseases of the Colon & Rectum*. 2001;44:706-12.
- [44] Zou Y, Brooks JL, Talwalkar V, Milbrandt TA, Puleo DA. Development of an injectable two-phase drug delivery system for sequential release of antiresorptive and osteogenic drugs. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*. 2012;100B:155-62.
- [45] Ke Y, Liu GS, Guo TH, Zhang Y, Li CH, Xue W, et al. Size Controlling of Monodisperse Carboxymethyl Cellulose Microparticles via a Microfluidic Process. *Journal of Applied Polymer Science*. 2014;131.
- [46] Wainwright M. Molds In Ancient And More Recent Medicine. *Mycologist*. 1989;3:21-3.
- [47] Halkes SBA, Van den Berg AJJ, Hoekstra MJ, du Pont JS, Kreis RW. The use of tannic acid in the local treatment of burn wounds: Intriguing old and new perspectives. *Wounds-a Compendium of Clinical Research and Practice*. 2001;13:144-58.
- [48] Barillo DJ. Topical antimicrobials in burn wound care: A recent history. *Wounds-a Compendium of Clinical Research and Practice*. 2008;20:192-8.
- [49] Clark A, Milbrandt TA, Hilt JZ, Puleo DA. Tailoring properties of microsphere-based poly(lactic-co-glycolic acid) scaffolds. *Journal of Biomedical Materials Research Part A*. 2014;102:348-57.

- [50] Zhu XH, Tabata Y, Wang CH, Tong YW. Delivery of Basic Fibroblast Growth Factor from Gelatin Microsphere Scaffold for the Growth of Human Umbilical Vein Endothelial Cells. *Tissue Engineering Part A*. 2008;14:1939-47.
- [51] Konig G, Hamlin BR, Waters JH. Topical Tranexamic Acid Reduces Blood Loss and Transfusion Rates in Total Hip and Total Knee Arthroplasty. *Journal of Arthroplasty*. 2013;28:1473-6.
- [52] Cervera C, Castaneda X, de la Maria CG, del Rio A, Moreno A, Soy D, et al. Effect of Vancomycin Minimal Inhibitory Concentration on the Outcome of Methicillin-Susceptible *Staphylococcus aureus* Endocarditis. *Clinical Infectious Diseases*. 2014;58:1668-75.
- [53] Young S, Wong M, Tabata Y, Mikos AG. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *Journal of Controlled Release*. 2005;109:256-74.
- [54] Chiang HY, Herwaldt LA, Blevins AE, Cho E, Schweizer ML. Effectiveness of local vancomycin powder to decrease surgical site infections: a meta-analysis. *Spine Journal*. 2014;14:397-407.
- [55] Mobbs RJ, Sivabalan P, Li J. Minimally invasive surgery compared to open spinal fusion for the treatment of degenerative lumbar spine pathologies. *Journal of Clinical Neuroscience*. 2012;19:829-35.
- [56] Ialenti MN, Lonner BS, Verma K, Dean L, Valdevit A, Errico T. Predicting Operative Blood Loss During Spinal Fusion for Adolescent Idiopathic Scoliosis. *Journal of Pediatric Orthopaedics*. 2013;33:372-6.
- [57] Champion JA, Walker A, Mitragotri S. Role of particle size in phagocytosis of polymeric microspheres. *Pharmaceutical Research*. 2008;25:1815-21.
- [58] Cannon GJ, Swanson JA. The Macrophage Capacity For Phagocytosis. *Journal of Cell Science*. 1992;101:907-13.
- [59] Parsian AR, Vatanara A, Rahmati MR, Gilani K, Khosravi KM, Najafabadi AR. Inhalable budesonide porous microparticles tailored by spray freeze drying technique. *Powder Technology*. 2014;260:36-41.
- [60] Bormann N, Schwabe P, Smith MD, Wildemann B. Analysis of parameters influencing the release of antibiotics mixed with bone grafting material using a reliable mixing procedure. *Bone*. 2014;59:162-72.
- [61] Laila L, Febriyenti F, Salhimi SM, Baie S. Wound healing effect of Haruan (*Channa striatus*) spray. *International Wound Journal*. 2011;8:484-91.
- [62] Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Advanced Drug Delivery Reviews*. 2012;64:72-82.

[63] Imani R, Rafienia M, Emami SH. Synthesis and characterization of glutaraldehyde-based crosslinked gelatin as a local hemostat sponge in surgery: An in vitro study. *Bio-Medical Materials and Engineering*. 2013;23:211-24.

[64] Matsumot.T, Hardaway RM, Dobek AS, Noyes HE. Antibiotic Topical Spray Applied In A Simulated Combat Wound. *Archives of Surgery*. 1967;95:288-&.

[65] Galland RB, Karlowski T, Midwood CJ, Madden MV, Carmalt H. Topical Antiseptics In Addition To Peroperative Antibiotics In Preventing Post-Appendectomy Wound Infections. *Annals of the Royal College of Surgeons of England*. 1983;65:397-9.

VITA

Place of birth

- Surrey, England

Educational institutions attended and degrees already awarded

- Bachelors of Science, Biomedical Engineering,
The Ohio State University, Columbus Ohio

Professional positions held (do not include job descriptions)

- Research Assistant, Regenerative Biomaterials
Laboratory, University of Kentucky

Typed name of student on final copy

- Nicholas John Andersen